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CHALLENGES OF THE ONGOING COVID-19 PANDEMIC

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Challenges of the ongoing COVID-19 pandemic

Guest Editors: Gregory Walker and William Rawlinson

Contents

Cover image: Background image: immunofluorescent uninfected (blue) and SARS-CoV-2 delta infected (red) Vero E6 cells (courtesy of Dr Stuart Hamilton, NSW Health Pathology and UNSW). Inset photographs: postdoctoral scientists from UNSW performing live-SARS-CoV-2 work in the Kirby Institute's Physical Containment Level 3 Laboratory.

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Vertical Transmission

Mark Schembri
President of ASM

Greetings everyone in my first Vertical Transmission for the new year. I can't believe we are already in March! ASM Hour has kicked off and I hope you joined the first two sessions, which featured talks on Public Health Microbiology Challenges in the Australia–Pacific region (hosted by Lea-Ann Kirkham and Robyn Marsh) and from our new Gut Microbes Special Interest Group (hosted by Christine Ong and Sarah Revitt-Mills). Hayley Newton (VP Scientific Affairs) and our theme leaders have assembled a great list of topics and there are fantastic talks lined up. We will continue to use the same link for all ASM Hour sessions (see here), which should make these national invited talks accessible to all of our membership.

We recently celebrated International Women's Day, and I would like to acknowledge the valuable and significant contribution that women make in the Australian Society for Microbiology (ASM). Our Executive is committed to providing equal opportunities for women in the ASM, guided by our new Equity, Diversity and Inclusion committee. The EDI committee, chaired by Dr Yogitha Srikhanta, is already actively contributing to ASM decision processes.

I would like to take this opportunity to remind you about our Communication Ambassador Program. Our ambassadors contribute to ASM's communication channels by sharing content on ASM events, showcasing their work, and serving as public advocates for microbiology. We provide all ambassadors with professional communication training from Science Technology Australia covering social media strategies, fostering a personal brand online, and becoming an online influencer in science and beyond. If you are an early-career researcher and interested in joining this program, applications for this year are open until 31 March and a link to the application form is available here.

This year is jam-packed with great ASM conferences, so please check out our general, specialised and co-supported meetings on our website. This includes our co-supported Parasitology MasterClass 2024 (April), Viruses of Microbes 2024 (July) and Biomolecular Horizons 2024 (September) meetings, and our specialised EduCon 2024 and CliniCon 2024 (both in July), AusME 2024 (October) and BacPath 2024 (November) meetings. Keep an eye out for discounted registration costs for ASM members to attend these meetings. Our highlight Annual Scientific Meeting in Brisbane is also approaching fast (1–4 July). Registrations and abstract submissions are open. I encourage everyone planning to attend the conference to book your travel early, as the cost of flights may rise quickly. We have a fantastic list of International Plenary speakers, as well as feature presentations from Prof. Matthias Boll (Germany; Rubbo Orator), Prof. Jessica Blair (UK; Snowden Orator) and our own Prof. Mark Walker (ASM Distinguished Orator Award winner). I hope to see you there!

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Microbiology Australia

Contextualising COVID-19 in 2024

Gregory Walker and William Rawlinson

The COVID-19 global pandemic, due to SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus type 2) has infected over 750 million people globally since November 2019. The majority of the Australian population (estimates of over 65% of the total) have been infected. The first diagnosed Australian case was on 25 January 2020 in a person returning from the source of the pandemic in Wuhan, Hubei Province, PR China. The global pandemic was declared on 11 March 2020, following the WHO's declaration of a public health emergency of international concern (PHEIC) on 30 January 2020. Initial control efforts in Australia focused on suppression strategies, which, by July 2021, were moved to less-suppressive and more-outbreak control strategies. This occurred alongside significant developments in vaccination, antiviral therapies, and increased percentages of the population becoming infected. Since then, more than 22 million Australians have been vaccinated, and the number of new COVID-19 cases and associated deaths decreased considerably, although outbreaks throughout the year (rather than only in winter) have continued to occur to date.

As we continue to navigate COVID-19, the challenges are persistent and evolving. Extensive resource allocation in the early pandemic response led to significant understanding of SARS-CoV-2, as well as advances in many adjacent fields. Now, as we approach 4 years since the onset of COVID-19, the continued burden highlights the critical importance of adaptive and forward-thinking approaches across basic science and public health. This issue of *Microbiology Australia* showcases the wide-ranging expertise of internationally recognised Australian researchers who continue to innovate in this area. We are delighted to present research on epidemiology,[1] next generation surveillance of viruses,[2] and the changing virology of SARS-CoV-2.[3] Long COVID has emerged as a significant clinical burden,[4] and there are several therapeutic developments in the pipeline to tackle this issue.[5] Advances in vaccine development have been crucial in the fight against COVID-19.[6] Continued investment in this field will ensure that we are prepared to tackle future pandemic threats,[7] with a 'One Health' approach at the centre of future public health strategies.[8]

So, we feel that providing up-to-date summaries, and data from experts in the area of SARS-CoV-2 infection, acknowledging that there are now many people in Australia who could write similar reviews, will provide a basis for wider discussion and review for Australian microbiologists. The likelihood of future re-emergence of other novel viruses, including zoonotic-derived coronaviruses, means that we must avoid complacency. The practice of infection control, One Health and virology has been changed irrevocably by the COVID pandemic. The World Health Organization was quoted in 2022 as stating, 'the COVID-19 pandemic taught us that strong, high-quality health systems must reach everyone'.[9] This global interdependence of pandemics is important to remember in going forward. We hope that the expertise and scholarship provided by the authors in the current issue of *Microbiology Australia* assist in assessing where we are now, and thinking about the future in relation to COVID-19 and future pandemics. Further, the long-term effects of COVID-19 still need to be assessed and large-scale, well-constructed studies with carefully defined outcomes need to continue.[10] It will be through such well-constructed studies that more-definitive answers can be obtained to guide our understanding of short-term responses, and long-term consequences, informing our ongoing research.

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The evolving epidemiology of SARS-CoV-2

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ABSTRACT

Pandemics usually start with a bang following the emergence of a new pathogen that is both sufficiently infectious and virulent to pose a substantial threat and warrant an emergency response. The very fact that a pandemic involves a new or substantially changed infectious agent means the initial response is hampered by limited epidemiological data and a large amount of uncertainty. It was in this context that COVID-19 infections spiralled in many countries in early 2020, overwhelming health systems and driving excess mortality. Without reliable data it was initially unclear who was most at risk of, and from, infection, or of transmitting the virus to others. Over the course of the pandemic global research efforts have gradually pieced together the complex epidemiology of SARS-CoV-2 infections and longer-term sequelae, but there is still much work to be done. The situation also continues to evolve as the virus mutates, public health responses change, effective treatments become available, and population level immunity is acquired and matures. Although the onset of a pandemic is usually explosive and undisputed, the end is rarely as dramatic or as certain. Tracking the changing epidemiology of SARS-CoV-2 in the transition from pandemic to endemic is essential and remains a significant task.

Keywords: COVID-19, disease control, epidemiology, infectious disease, pandemic preparedness, public health, SARS-CoV-2.

Introduction

The epidemiological definition of ‘pandemic’ is actually quite general: ‘an epidemic occurring over a very wide area, crossing international boundaries and usually affecting a large number of people’ (p. 209).[1] However, the defining characteristic of a pandemic that prompts a coordinated global emergency response is the combined threat of rapid transmission, simultaneous outbreaks in multiple countries, and severe illness and high fatality rates.[2] This is most likely to occur with the emergence of a new pathogen, or where there has been a substantial change in a pathogen that renders existing population immunity ineffective. Pandemics end when the disease is brought under control – either eradicated, which is rarely possible, or it becomes endemic as the pathogen and humans co-adapt.

The SARS-CoV-2 virus, and the COVID-19 disease it causes, was first reported in early 2020, though it likely was causing diseases some months earlier,[3] and marked the beginning of the first truly global pandemic of the 21st century.[2] Although we had previously had to contend with SARS and MERS which were caused by related coronaviruses,[4] this new coronavirus presented key epidemiological differences that would affect transmission dynamics and our ability to control and contain the morbidity and mortality that would follow.

The main routes of transmission of the coronaviruses causing COVID-19, SARS and MERS appeared similar in the very early stages of the pandemic (aerosol transmission detection increased with later SARS-CoV-2 variants), but SARS and MERS had higher case fatality rates (~10 and 35%, respectively) compared with COVID-19 (averaging 2.5% in the pre-vaccine phase[5]). Global case fatalities estimates ranged from 1.7 to 39.0% in February–March 2020, falling below 0.3% by August 2022, a relative risk reduction of 96.8% over 2.5 years (95% confidence interval 95.6–97.6[6]).

However, it was the efficiency of transmission of SARS-CoV-2 and the resultant high infection rates globally that led to an estimated seven million deaths attributed to COVID-19 at the time of writing (reported at https://www.worldometers.info/coronavirus/, accessed 15 January 2024) compared with the substantialy lower total global reports for deaths from MERS (806 deaths) and SARS (774 deaths).[5] Fundamental

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differences in the epidemiology among these coronaviruses (length of incubation period, viral load and when it peaks, infectious period commencing before symptoms, variability in infection and severe disease susceptibility, case fatality rates and so on) had to be understood to assess the potential need for, and likely effectiveness of, public health interventions. SARS-CoV-2 also stands out from SARS and MERS as it has not only spread to all countries but has also persisted within most populations for 4 years or more. Over this time, key aspects of the epidemiology have changed with the succession of variants and subvariants and the development of population immunity.

The inevitable path to endemicity

SARS-CoV-2 is not amenable to eradication globally, or elimination at a local level, as we do not have a sterilising vaccination, natural immunity following infection wanes, and transmission can occur before or in the absence of symptoms limiting the effectiveness of public health measures.[7] Furthermore, there are multiple animal reservoirs that can seed reintroductions into the human population and accelerate the evolution of new subvariants in the cross-species spillover process.[8] Endemicity is the only other option, and is defined as 'the constant occurence of a disease, disorder, or noxious infectious agent in a geographic area or population group; it may also refer to the chronic high prevalence of a disease in such area or group' (p. 92).[1]

One of the ongoing misunderstandings is what endemicity means in practical terms. When a disease is classified as 'endemic', it does not imply that it not a significant or ongoing public health threat, nor does it preclude waves where infection rates spike or localised outbreaks occur. Rather, it signals that we have shifted from the emergency phase of the disease response, usually focussed on dampening community transmission to protect lives and prevent health system overload while vaccines and treatments are developed and distributed, to managing the disease in a sustainable way given the likelihood of persistence.[9] From late 2021, countries that had previously tightly managed international boarders to limit transmission began to open, including Australia. By early 2023, it was widely acknowledged that the world was transitioning to an endemic phase, with the World Health Organization (WHO) declaring the emergency phase over in May of that year.[10]

From dominant variant of concern to omicron subvariant soup

As SARS-CoV-2 established itself globally during the COVID-19 pandemic, we saw a succession of waves driven by the emergence of new variants with different combinations of mutations that, at various times, rendered the virus more or less virulent, more transmissible (generating higher viral load, binding affinity, upper respiratory infection sites) or instilled immune escape advantages (for example, changes to the spike protein increasing immune escape ability).[11] Most original COVID-19 waves were dominated by a single new variant, but by late 2021 when Omicron emerged, we saw a new pandemic phase with this single Variant of Concern dominating across waves, and multiple Omicron subvariants emerging, co-existing and persisting in communities.

The rate of transition from one SARS-CoV-2 variant to the next has been shown to be associated with vaccination rates, number of co-circulating variants, and convalescent immunity.[12] A mix of subvariants circulating concomitantly also creates a more complex epidemiological picture, further compounded by variable levels of immunity, vaccination- and infection-derived, within and across populations, and variable susceptibility to severe disease depending on age, comorbidity and immunity.[12]

Heterogeneity in infection and disease risk within populations and over the course of the pandemic challenges risk quantification and communication as the risk itself becomes more individualised and population averages less informative. The estimation of risk is further complicated when rapid transmission of a pathogen through the population, or rapid vaccine and treatment rollouts, makes it difficult to capture important comparative data from individuals before they are exposed. This is particularly important for comparison studies of acute and long-term disease risk in pandemics where the disease profile is variable in both severity and the symptoms that present, and the symptoms overlap those of pre-existing conditions.

Even in epidemiologically stable contexts, risk can be very challenging to communicate during a public health emergency and there can be significant consequences for public adherence to public health measures if risks are communicated poorly. Risk is more than the epidemiological likelihood of an infection occurring or the calculation of the effectiveness of an intervention containing transmission, it is a construct that draws together notions of hazard and outrage, and it is therefore essential that risk communication is inclusive and engages with the population where these views are formed and acted upon.[13]

The end of population-wide transmission control measures

In 2020, the ancestral variants required stringent public health measures, including international border closure, lockdowns and behavioural and mobility restrictions, to successfully limit community transmission to the levels required for disease control in an immunologically naïve population.[14] With increasing transmissibility and immune escape properties of subsequent variants, and shorter incubation periods, these same control measures have become less effective, particularly with the emergence of the highly transmissible Omicron variant and successive subvariants.[15]

Adherence to population level public health orders also cannot be assumed to be an endless commodity to call upon, especially among those who have been informed they are less at risk of severe illness or have experienced mild illness previously.[16] Non-pharmaceutical interventions also carry risk of other negative effects on health, healthcare access, population health and health inequalities,[17] and so the public health policy risk calculus supporting interventions shifts as the

risks associated with infection reduce with increasing population immunity, and treatment options expand and improve.

The range of symptoms that can present in a COVID-19 infection, together with variability in severity and time to onset of symptom, make it hard to know exactly where the virus is circulating in the community. This is further exacerbated when infections remain symptom-free throughout. All have become more common with the emergence of Omicron, and in highly immune populations,[18] making the identification and management of infections, and of people in their infectious period, more challenging. A study of 210 people who tested positive on serology indicating a recent SARS-CoV-2 infection during the Omicron era reported that the majority (56%) were unaware that they had been infected,[19] compared with 44% in a large US population-based sample from early 2022,[20] and an estimated 35% of cases early in the pandemic.[21] More recently, data from late 2022 to January 2023 were combined from three prospective US cohort studies with 2959 participants with active testing not subject to biases associated with symptom presence or severity. Of the 426 infections discovered, 56.8% were asymptomatic, and symptomatic infections were mainly reported among those either not vaccinated, or who had only received the original monovalent vaccine.[22]

Recent research[23] has also shown that viral load rises later, on average, during Omicron infections compared with earlier in the pandemic where the highest viral load in throat swabs was reported at the time of symptom onset, and the inferred infectiousness peaked on or before symptom onset.[24] The viral load in Omicron infections was found to most commonly peak on the third or fourth day after symptoms developed.[23] This might seem epidemiologically beneficial if cases are less likely to be at their most infectious before symptoms emerge and can manage their risk of exposing others to the virus. However, it also means that Rapid Antigen Tests (RAT) are now more likely to generate false negative results even a few days into the symptomatic period.[23]

Earlier in the pandemic, a single negative RAT taken soon after symptoms developed had a meaningful negative predictive value, but with later viral load peaks and reduced RAT sensitivity until a few days into the symptomatic period, a negative result may lead people with COVID-19 to wrongly conclude that their symptoms must be due to another cause. What disease control benefits there may be in cases having more time to register that they have an infection before they reach peak infectiousness is lost if symptoms are so mild or vague that they are missed or not recognised as COVID-19. Asymptomatic infection, mild signs and symptoms or symptoms not readily recognisable as COVID-19 are all likely to result in more cases failing to recognise and manage their infection. This will only be further exacerbated if there is a higher risk of false negatives on RATs taken, even after symptoms appear. Individuals at high risk of severe disease, and their close contacts, now need to increasingly rely on repeat RAT testing to manage exposure risk.

Conclusion

The WHO announcement on 5 May 2023 declaring that COVID-19 was no longer an international public health emergency[10] officially signalled the SARS-CoV-2 transition from pandemic to endemic. Endemic diseases that pose a significant health burden, such as COVID-19, remain a public health priority and require ongoing surveillance and application of analytical methods to address the ever-increasing complexity of the epidemiology that occurs in the transition. Surveillance and analysis are vital in tracking local and global changes in epidemiology, and providing reliable estimates of risk to inform public health responses and support effective public health messaging to keep populations informed and actively engaged in health protection.

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Biographies

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Assoc. Prof. Hassan Vally is an epidemiologist with considerable experience in both academia and in government. He has expertise in the analysis and interpretation of health data, and in the understanding and critiquing of evidence in the health domain. He has background in a number of disciplines in addition to epidemiology, including molecular biology, virology and immunology. Dr Vally is an expert in risk and risk communication and has been involved in consulting for both the State and Federal Governments as well as the World Health Organization. In recent years, Dr Vally has been heavily involved in science communication and has been actively involved in media engagement.

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Wastewater-based SARS-CoV-2 surveillance and sequencing

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ABSTRACT

Though most often associated with poliovirus surveillance, wastewater-based surveillance (WBS) can be employed for viruses shed in human excreta (faeces, urine, skin, sputum, blood) that may enter the wastewater system, including SARS-CoV-2. WBS has been widely adopted during the COVID-19 pandemic, to complement clinical surveillance in monitoring community burden and implementing timely public health interventions. As wastewater is a non-biased, composite sample, it can provide population-level health information in near real-time, in a cost-effective manner compared to similarly scaled clinical surveillance. In many instances, data gathered from wastewater, including viral loads (quantified by RT-qPCR) and variant detections (determined through partial or whole genome sequencing), have been predictive of what is observed eventually in clinical cases. Newly emergent lineages, including the recent BA.2.86 variant, can and have been detected in wastewater samples prior to their detection in clinical specimens. There remain many challenges to wastewater genomic analysis including the presence of RT-qPCR inhibitors, degraded nucleic acid and the lack of consistent or standardised methodology between reporting labs. The wide adoption of WBS practices provides an excellent opportunity to expand this method for surveillance of other pathogens of public health importance. Herein, a broad overview of the WBS field will be provided including discussion on its advantages and applications, challenges, and how it is being utilised to characterise circulating SARS-CoV-2 lineages through sequencing.

Keywords: COVID-19, next generation sequencing, public health, sewage, viral pathogens, virus surveillance, wastewater-based epidemiology.

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Analysis of wastewater for the surveillance of human pathogens, such as poliovirus, has been implemented and refined over the past several decades. Wastewater-based surveillance (WBS) can broadly involve the analysis of sewage for pathogen nucleic acid and other biomarkers that are excreted in human waste to provide near-real time population-level health information. Historically, WBS has been used not only for enteric virus surveillance, but also for the monitoring of community illicit drug use,[1] alcohol and tobacco use,[2,3] and for the monitoring of antimicrobial resistance genes.[4]

A spectrum of viruses may enter the wastewater system through sputum, skin, blood, faecal and urinary secretions including respiratory viruses such as influenza virus[5] and respiratory syncytial virus,[6] blood-borne viruses and vector-borne viruses.[7] The utility of wastewater as a surveillance tool has been widely and rapidly adopted with the emergence of severe acute respiratory syndrome 2 virus (SARS-CoV-2) in 2020, as a means to mitigate community spread, monitor prevalence and more recently, to characterise circulating lineages. A simple overview of the wastewater surveillance process is presented in Fig. 1.

Wastewater-based surveillance for SARS-CoV-2

It has been almost 4 years since the declaration of the Coronavirus disease 2019 (COVID-19; caused by infection with SARS-CoV-2) pandemic in March 2020.

During the early stages of the COVID-19 pandemic, it was established that SARS-CoV-2 RNA was detectable and quantifiable in the faeces[8,9] and urine[10] of infected individuals. The ability to detect and quantify SARS-CoV-2 RNA in municipal wastewater was also established in multiple countries in the early months of 2020 including Australia,[11] USA,[12] Netherlands[13] and Japan.[14] In the Netherlands, for example, RNA targets were

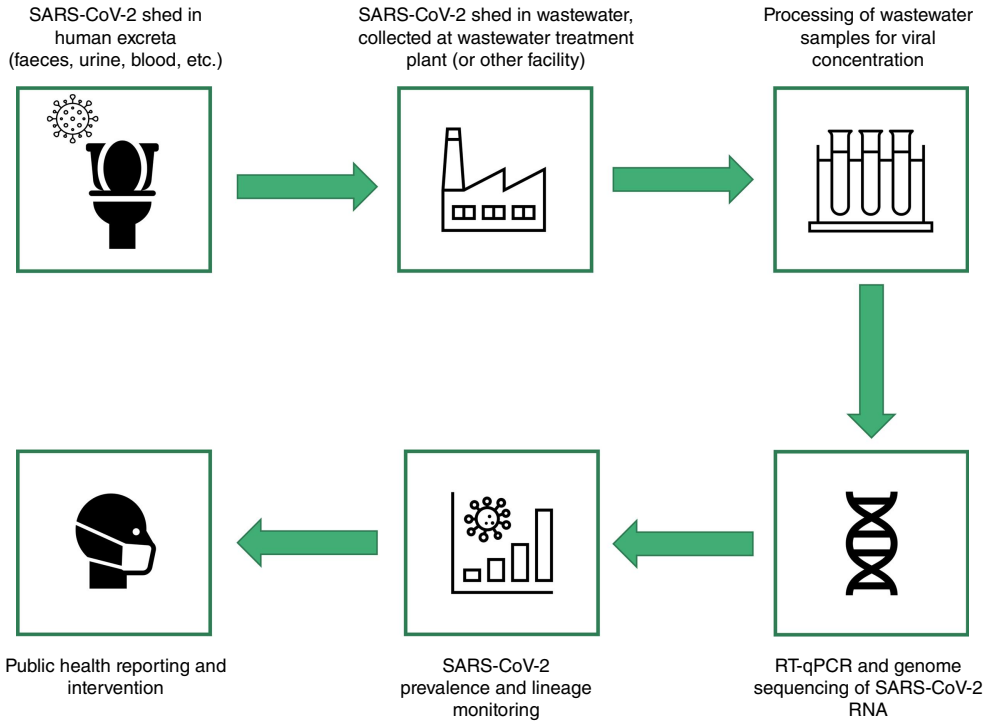

Fig. 1. A broad overview of the wastewater surveillance pipeline for SARS-CoV-2. Virus shed from human secretions such as faeces, urine, sputum and blood may be captured in domestic wastewater. Wastewater from domestic sites is transported to wastewater treatment plants, where it may be collected for analysis. The method and frequency of collection can vary, and may involve a singular grab sample, or a composite sample collected at intervals over a defined period. Collected samples are transported to a lab for SARS-CoV-2 concentration and RNA extraction. RT-qPCR for SARS-CoV-2 RNA quantification may then be performed, as well as whole genome sequencing, either through direct sequencing (e.g. amplicon-based sequencing) or through a metagenomic approach. Trends in viral load and estimated relative lineage abundances observed in wastewater samples can then be reported to the relevant government body to inform public health interventions.

detected in the wastewater in the city of Amersfoort 6 days prior to the first clinical case detection.[13]

SARS-CoV-2 RNA detectable in wastewater may be shed from symptomatic and post-symptomatic individuals as well as the asymptomatic and pre-symptomatic.[15] Information from the latter would ordinarily be underreported through traditional clinical case surveillance, making wastewater surveillance an effective complement to clinical detections.

The composite nature of wastewater provides the opportunity to obtain SARS-CoV-2 data at a community level, in a more cost-effective manner compared to similarly scaled clinical testing. The capture of virus shed from the asymptomatic and pre-symptomatic often allows for the foreshadowing of case trends and outbreaks prior to observation by clinical surveillance alone.[16] In Montpellier, France, for example, a post-lockdown surge in cases in late June 2020 occurred ~2–3 weeks after an observed increase in viral RNA levels in wastewater.[17] Wastewater is a non-biased sample that captures virus shed by anyone in a community connected to a sewage system, independent of healthcare seeking behaviour and access to clinical testing. In resource-poor settings and countries that have limited infrastructure for large-scale clinical testing, WBS is an especially useful strategy for understanding burden and prevalence.[18] WBS may be applied at the level of individual, high risk institutions including university campuses, hospitals, cruise ships, aged-care facilities, school, airports, prisons and populations with limited healthcare access.[19–23]

Throughout the pandemic, WBS has been used in complement to traditional surveillance methods, to address the public health priorities of the time. Early in the pandemic, when many jurisdictions were aiming for zero community transmission, WBS focused on viral RNA detection and RT-qPCR quantification to alert of new cases in a community and to monitor trends, which were often predictive of clinical case data.[16] Information from WBS was used to guide public health interventions, usually in the form of social restrictions. As community transmission initially became more widespread, there remained strong community willingness to present for nucleic acid based testing and strong government incentive to monitor case numbers.[24] During this phase, WBS remained focused on RT-qPCR quantification to monitor case burden, but also shifted to include lineage characterisation through partial or whole genome SARS-CoV-2 sequencing. This was a complement to the extensively available clinical sequence data and was used to monitor trends and incursions of lineages of concern. As at-home rapid antigen self-testing (RATs) became more widely adopted, there was less clinical lineage data made available. With issues regarding RAT sensitivity and their correct use and interpretation, as well as decreased willingness to perform and report testing, there was a progressive

reduction in the understanding of true community disease burden.[24] During this latter phase of the pandemic, shifting towards a state of endemicity with high community vaccination rates, there has been greater reliance on wastewater data to supplement missing clinical data, including information on circulating SARS-CoV-2 lineages and their estimated relative abundance. Today, routine SARS-CoV-2 wastewater monitoring has been adopted in many jurisdictions in over 50 countries, including in most states and territories of Australia.[24]

Challenges of wastewater-based surveillance

Wastewater is an innately complex matrix. Not only does municipal wastewater contain waste from household settings, it also includes waste from industrial, agricultural and retail sources. As such, sewage can contain many substrates inhibitory to RT-qPCR including detergents, metal ions, polysaccharides, RNases and is subject to temperature fluctuations.[25] Nucleic acid is often fragmented in wastewater, making sequencing efforts challenging.[26] There is great variability between samples collected at a single site (e.g. turbidity and presence of inhibitors), and more variability between samples from different sites.[27]

Additionally, despite the global expansion of WBS, there is no single, standardised and accredited method for the concentration, quantification and sequencing of viral RNA from treated or untreated wastewater.[25] There are many steps involved in wastewater processing, with many deviations at each. Common methods employed for virus concentration include polyethylene glycol (PEG) precipitation, ultrafiltration, ultracentrifugation and adsorption-extraction.[7] When designing a wastewater surveillance program, there needs to be consideration of the sampling method, frequency, collection volume and the target catchment to capture a representative population of appropriate size.[25] Consideration for the fraction of wastewater (i.e. the solid or aqueous phase) to be tested is also required, based on the partitioning behaviour of the target viruses. Generally, enveloped viruses are sequestered in the solid phase, whereas non-enveloped viruses are poorly adsorbed to solids.[28] Although there have been efforts to review and compare wastewater processing strategies, there remains a lack of harmony in the methods used between jurisdictions, which may affect how we interpret data and how it can be shared and utilised.[27]

SARS-CoV-2 lineage surveillance through wastewater sequencing

Throughout the COVID-19 pandemic, many SARS-CoV-2 variants have emerged, including variants of concern (VOCs) and interest (VOIs), which have been important to identify and monitor. Genomic surveillance through the sequencing of SARS-CoV-2 RNA from wastewater samples has allowed cost-effective elucidation of variant distribution and spread, and early emergent lineage detection.[29]

Sequencing from wastewater poses several challenges. PCR inhibitors present in wastewater, as well as fragmented template makes it challenging to derive good quality sequence data of satisfactory coverage.[25] Perhaps the most challenging aspect of wastewater sequencing is data interpretation, and delineating variant abundance from a sample that would contain various lineages shed from multiple infected individuals.[29] Most standard tools and programs for SARS-CoV-2 lineage designation were devised for typing of a single, clinical infection, rather than a mixed environmental sample. The Freyja tool has become one of the most widely adopted methods for delineating the relative abundance of lineages within a wastewater sample.[29] It uses a library of lineage-defining mutational barcodes of data from the global phylogenetic tree UShER (ultrafast sample placement on existing trees) to derive relative lineage abundances.[29] An example Freyja analysis output is presented in Fig. 2, presenting the relative abundance estimates of major parental lineage groups detected in two wastewater samples collected 2 weeks apart from the same wastewater treatment plant in Sydney, Australia, between October and November 2023.

SARS-CoV-2 wastewater sequencing (including partial and whole genome sequencing) has been used to successfully detect and track emergent lineages, occasionally prior to their detection in clinical cases in that community or institution.[29,30] The recently emerged BA.2.86 variant, for example, was detected in wastewater 1 week prior to detection in clinical sampling, in the Stockholm region,[31] whereas early detections of Omicron (B.1.1.529) in wastewater were made in several American localities.[30,32] The application of viral metagenomics to wastewater provides an opportunity for the identification and monitoring of known and unknown pathogens, and, in past studies, has identified a diversity of viral families including *Coronaviridae*, *Flaviviridae*, *Poxviridae*, *Adenoviridae*, *Herpesviridae* and *Retroviridae*.[7]

Metagenomics is an appealing strategy to derive abundant information from a single test, though is challenged by the high magnitude of background bacterial and fungal sequence data, and challenges in deriving high quality viral sequence data.[7]

Conclusions

Wastewater surveillance provides a cost-effective, non-biased, non-invasive surveillance tool that can complement traditional clinical surveillance practices, and potentially provide forewarning of imminent outbreaks and introduction of new variants into a population.

Wastewater surveillance for SARS-CoV-2 has been an invaluable tool over the many phases of the COVID-19 pandemic for informing public health interventions and stemming community spread. In the current phase of the pandemic, where we have shifted towards endemicity with massively reduced clinical nucleic acid-based testing, wastewater has become an essential tool for understanding the diversity of lineages that are circulating in the community, and their dynamics. The wide-spread adoption of wastewater surveillance practices in more labs than ever before, has established an infrastructure that can readily incorporate the surveillance of other pathogens of public health importance.

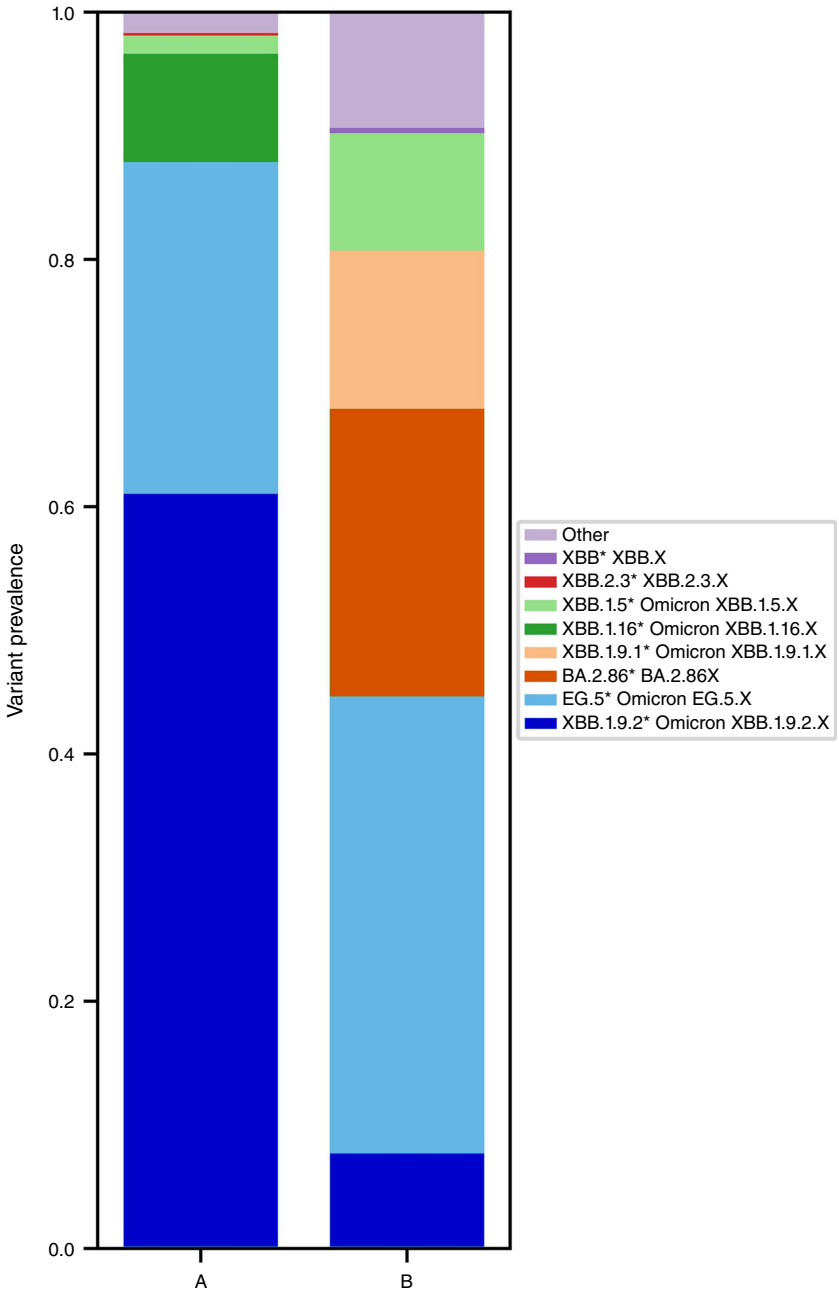

Fig. 2. An example plot that may be generated with the output of a Freyja analysis on wastewater data. The figure shows the estimated relative abundance of two samples (A and B) collected from the same sampling site in Sydney, Australia. Sample A was collected in mid-October 2023, and sample B was collected in early November 2023, ~2 weeks apart. The estimated relative abundance (as a proportion of 1.0) of summarised 'parental' lineage groups is presented, as per the colouring in the key. Comparing the results of the two sites, a change in lineage abundance can be observed, particularly for lineage groups XBB.1.9.2* and BA.2.86*. The 'Other' lineage group in these examples includes non-XBB* and non-XBC* recombinant lineages.

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Biography

Dr Alice Michie is a postdoctoral scientist at New South Wales Health Pathology. She currently performs whole genome sequencing of SARS-CoV-2 of NSW wastewater samples from sentinel sites.

Call for Early Career Research articles

There has been a recent decision to allow 1–2 articles in each issue from Early Career Researchers who are ASM members. This will replace our biennial ECR issue of *Microbiology Australia* and provide more flexibility for those who are ready to publicise their research and research area. Papers are generally of the In Focus or Lab Report type. Guidelines are available from the journal website or the Editor.

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Cellular signalling by SARS-CoV-2 spike protein

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ABSTRACT

Following the release of the SARS-CoV-2 genome, the spike protein was identified as the key viral protein mediating cell entry. In addition to its critical function in delivering the viral genome to the host cytoplasm, the spike protein is able to activate diverse cell signalling pathways, leading to notable cellular responses, including inflammation, cellular remodelling, and immune evasion. The spike protein is associated with the induction of a 'cytokine storm' characterised by elevated levels of proinflammatory cytokines like IL-6 and IL-1β. Moreover, the spike protein deregulates TGF-β and E-selectin, leading to fibrotic injury and tissue scarring in cellular remodelling, notably in pulmonary tissues. Finally, the spike protein plays a role in immune evasion, disrupting Type I interferon responses. Understanding these diverse interactions and effects is crucial for comprehending the pathogenesis of COVID-19 and developing effective therapeutic strategies.

Keywords: ACE2, Angiotensin Converting Enzyme Receptor 2, cellular signalling, COVID-19, cytokine storm, fibrosis, spike protein, TGF-β, transforming growth factor-beta.

SARS-CoV-2, the viral cause of COVID-19, remains a global threat to human health. The spike protein is a glycoprotein encoded by SARS-CoV-2 that mediates membrane fusion and entry into host cells primarily by binding to ACE2. ACE2 expression is highly restricted to specific cell types and largely defines cells that are susceptible to infection although alternative ACE2 pathways have been described.[1–4] Current vaccination strategies are also based on delivery of the spike protein through various technologies, which have proven highly effective at reducing transmission, infection, hospitalisation and mortality.[5–7] Beyond cell entry and adaptive immune priming, the spike protein also modulates various host cell signalling pathways which ultimately contributes to the pathology and spread of the virus (Fig. 1). These effects can be seen as ACE2-dependent or ACE2-independent, suggesting the spike protein has roles outside of binding. For example, the spike protein is sufficient to induce highly inflammatory cytokines, which may contribute to the multisystem inflammatory syndrome, otherwise known as a 'cytokine storm' observed in COVID-19. In addition, the spike protein mediates key changes to the cellular microenvironment that contribute to fibrotic and vascular pathologies. Emerging evidence suggests the spike protein also plays a critical role in subverting host immune signalling to avoid host defences. This review will examine functions of the spike protein independent of ACE2-mediated entry, and how these contribute to COVID-19 pathology.

It is important to note that during infection, various subunits and forms of the spike protein can be released and presented to host cells to elicit differing effects (Fig. 2). The spike protein resides on the surface of SARS-CoV-2 virions as trimers. Spike-protein-mediated entry requires two critical cleavage events. Firstly, prior to infection and binding to ACE2, the spike protein is cleaved into subunits S1 and S2 by furin-like proteases. Subsequently, the cleavage of S2′ subunit by the cellular serine protease TMPRSS2, which results in delivery of the viral genome into the cytoplasm.[3,8–10] While the spike protein's role in entry by ACE2 has been extensively described, the modulation of cellular signalling by the spike protein, and its downstream effects, is an expanding area of interest. Here we describe three major cellular responses to the spike protein: inflammation, cellular remodelling and immune evasion.

Inflammation

In COVID-19, various proinflammatory cytokines and markers of tissue damage are elevated and associated with severe disease, infection complications and mortality.

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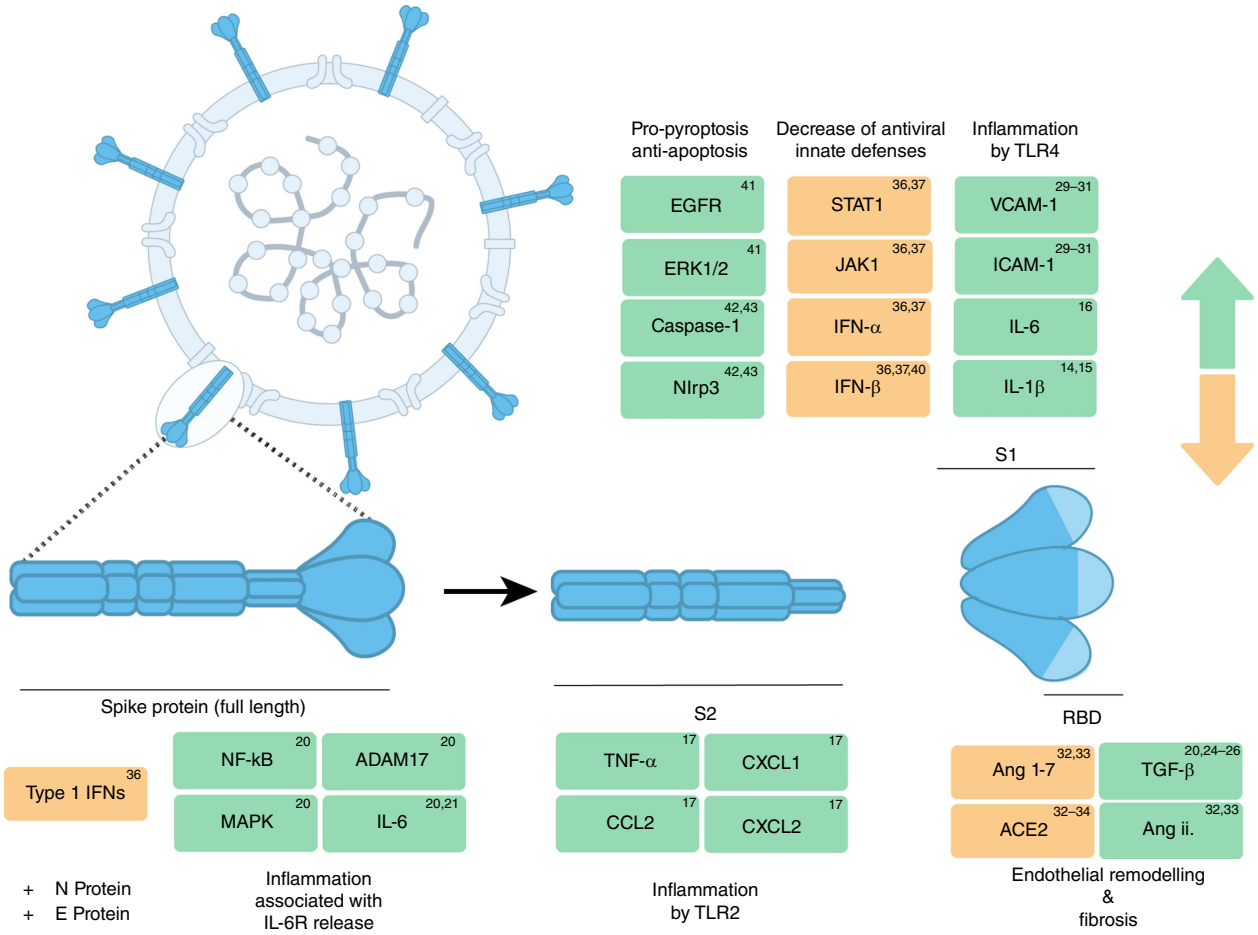

Fig. 1. Different spike protein subunits activate different forms of cell signalling. The spike protein is presented as a trimer on the surface of SARS-CoV-2 virions. The spike protein itself is divided into two subunits, S1 and S2, with S1 encompassing the Receptor Binding Domain (RBD), which initiates ACE2-dependent cell entry, and S2 containing the fusion peptide required for fusion with the host cell membrane. The furin-protease site located between the subunits is unique to SARS-CoV-2, absent in other viruses in the same clade.[11] Both full-length spike protein and its component subunits modulate different cell signalling pathways with diverse effects.

Such cytokines include, but are not limited to, IL-1β, IL-6, IL-10, TNF, and IFN-ɣ.[12] Secretion of proinflammatory IL-1β, IL-12 and TNF is mediated through the Pattern Recognition Receptor (PRR) Toll-like Receptor 4 (TLR4) and NF-κB.[12] Recent studies have identified that spike protein in isolation can bind to TLR4 and activate downstream NF-κB signalling, and ultimately drive expression of inflammatory effector genes.[13] Purified spike protein trimers directly bind TLR4 and induce IL-1β expression in a dose-dependent manner.[14] The spike protein S1 subunit interacts with TLR4, and in conjunction with IFN-ɣ, drives expression of proinflammatory IL-1β to induce the differentiation of proinflammatory M_1 macrophages *in vitro*.[15,16] Additionally, spike protein S1 subunit directly interacts with the Leucine-rich repeat domain of TLR4, and in a mouse model of cardiac infection, S1 subunit increased expression of IL-1β and IL-6 to induce inflammation.[17] A subsequent study identified a role for the spike protein S2 subunit in the induction of proinflammatory cytokines IL-6, IL-1β, TNF-α and chemokines CXCL1, CXCL2 and CCL2 in human and murine macrophages.[18] Here, TLR signalling was activated by the spike protein in TLR4 knockout macrophages, but not TL2 knockouts, suggesting a conflicting dependence on TLR2 rather than TLR4, contrary to previous studies.[18]

IL-6 has emerged as a key cytokine in driving a hyper-inflammatory state during COVID-19, especially in severe or complicated disease.[12] Although clinical trials have failed to show improvement from the use of IL-6 antagonists, it remains evident that the cytokine plays a key role in the development and progression of disease.[19] Administration of both polyI:C and the spike protein into ACE2-expressing mice found that IL-6 was induced nearly 100 fold.[20] Accordingly, transfecting the spike gene into epithelial cells results in induction of phosphorylated NF-κB, MAPK and secretion of IL-6.[21] Importantly, IL-6 *trans*-signalling may be a key contributor to inflammation-related pathologies.[22] During *trans*-signalling, soluble IL-6 receptors bind IL-6, forming a complex with gp130 that ultimately allows cells not expressing the IL-6 receptor (IL-6R) to respond to IL-6.[22] The spike protein has also been shown to activate the ADAM-17 protease, thereby releasing soluble IL-6R, which may increase IL-6 and IL-6R levels in COVID-19.[21] Furthermore, an *in vitro* *trans*-signalling model demonstrated that exposure to culture fluid from epithelial cells transfected with the spike protein was sufficient to induce IL-6 signalling in endothelial cells, which do not express transmembrane IL-6R.[21]

Taken together, these studies highlight the role of the spike protein in triggering rapid induction of inflammatory

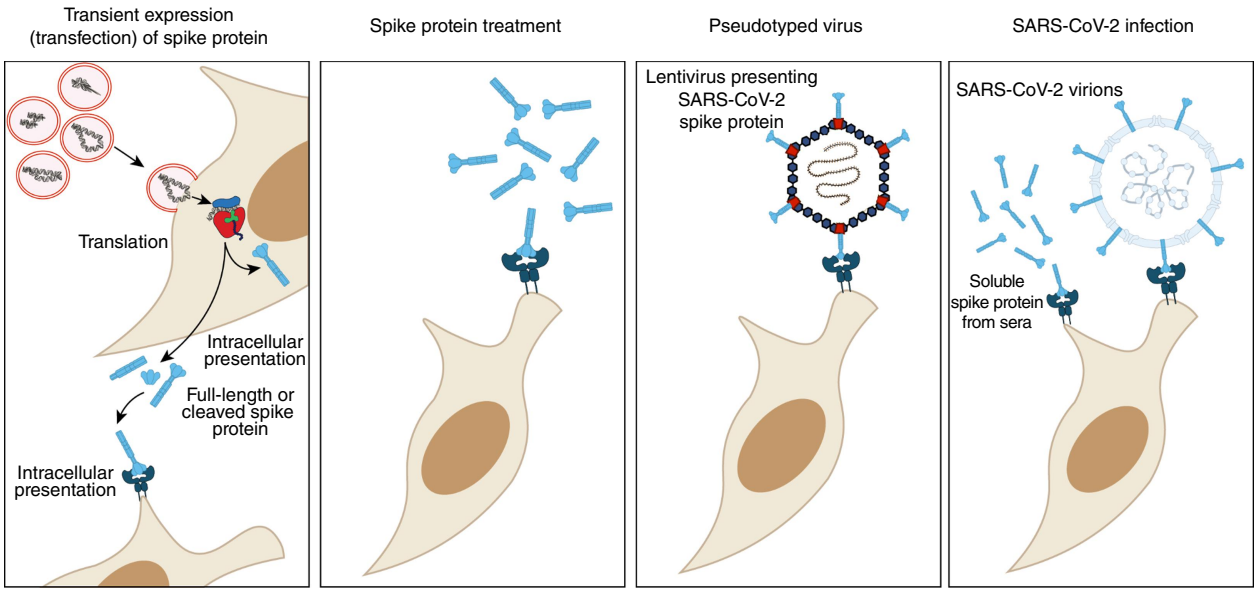

Fig. 2. Different modalities for investigating spike-protein activity. Cells are induced to express the spike protein *in vitro* by transfection or *in vivo* by mRNA nanoparticle complexes and adenovirus vectors. Post protein synthesis, spike protein forms trimers cleaved at the furin-protease cleavage site between S1 and S2 subunits.[23] Synthetic spike protein treatment and pseudotyped virus infection replicate the spike protein binding with ACE2. Live viral infection presents the spike protein to the cell surface and the intracellular environment during virus replication. Additionally, soluble spike protein is present in patients' sera for up to 12 months post-infection.[24]

effector genes and signalling molecules that are hallmarks of COVID-19 and correlate with disease severity.

Cellular remodelling

Major complications of COVID-19 include fibrotic injury and tissue scarring. Transforming growth factor-β (TGF-β) is considered the master regulator of fibrosis and tissue remodelling in both homeostasis and disease. In the context of COVID-19, TGF-β, along with multiple other pro-fibrotic cytokines, serves as a biomarker for lung injury.[25–27]

TGF-β has also been observed to cause endothelial barrier dysfunction following both full-length spike protein and the spike Receptor Binding Domain (RBD) treatment.[28] This spike-protein-induced barrier hyperpermeability, an effect observed previously with flavivirus non-structural protein 1 (NS1), has been speculated to promote viral dissemination and pathogenesis.[29] Furthermore, it was observed that spike-protein-induced endothelial barrier dysfunction *in vitro* and vascular leakage *in vivo*, driven by an upregulation in glycosaminoglycans (GAGs), integrin and TGF-β signalling.[28] Cellular remodelling in this study activated by the spike protein was also independent of ACE2. Pulmonary endothelial cell damage in COVID-19 is also tied to paracrine cell signalling. Similar to TGF-β, S1 subunit treatment activates VCAM-1 and ICAM-1 pathways, which contribute to cell vasculopathy. Spike-protein induction of VCAM-1 and ICAM-1 leads to increased expression of E-selectin and risk of blood clotting.[30–32]

Apart from its role as the key mediator for entry, ACE2 contributes to spike-protein-associated vascular remodelling and fibrosis. As a part of the renin–angiotensin system (RAS), ACE2 controls the hydrolysation of the peptide angiotensin ii to the less inflammatory and pro-fibrotic angiotensin(s) 1–7 during homeostasis. However, this balance is disrupted during vasoconstriction and fibrotic injury.[33] In the case of the S1 subunit interaction with ACE2 during viral entry, ACE2 is downregulated, generating an imbalance in the levels of angiotensin ii and leading to an inflammatory response, oxidative stress, vasoconstriction and fibrotic activity.[34,35] Similar to live viral infection, multiple methods of presenting the spike protein to cells, including full-length spike protein, ectopic expresison of S1 subunit, and pseudotyped virus infection, are shown to decrease ACE2 expression.[34,35] Notably, differences in ACE2 supression were observed between the two studies. Spike-protein treatment reduced ACE2 expression *in vitro* whereas both treatment and pseudotype virus-expression of the spike protein provided the same effect *in vivo*.[34,35] Additionally, angiotensin ii blockers increase viral replication in SARS-CoV-2 susceptible cell lines, presumably due to an increase in available ACE2.[36]

Immune evasion

The history of SARS-CoV-2 variants highlight the spike protein as the critical target in immune recognition and driver of enhanced transmissibility and infectivity. In addition, it is becoming increasingly evident that the spike protein itself is able to suppress host-cell Type I Interferon antiviral responses. Specifically, S1 subunit treatment activates the signal transducer and activator of transcription 1 (STAT1) to block association with Janus Kinase 1 (JAK1), leading to reduced IFN-α and IFN-β expression.[37,38] A separate study demonstrated that spike and membrane proteins cooperatively decreased IFN-mediated activation of NK cells while enhancing TGF-β activity.[39,40]

Ectopic expression of the spike protein reduces JAK-STAT activation of IFN activity in cells and spike gene transfection specifically blocks the RIG-1-induced activation of IFN-β with the aid of N protein.[41] Associated with this finding, cells transfected with the spike gene led to an increased susceptibility to infection by other RNA viruses.[37] IFN-α and IFN-β levels are characteristically low in severe COVID-19 patients compared to mild cases and the activity of spike protein to deregulate IFN production may explain this observation.[39]

Epidermal Growth Factor Receptor-Mediated (EGFR) signalling, a pathway known to crosstalk with TGF-β, is upregulated following treatment with S1. EGFR signalling also activates ERK1/2 and AKT kinases and enhances the expression of Survivin, a critical inhibitor of apoptosis.[42] Further, the spike protein exhibits anti-apoptotic activity in hematopoietic stem cells, triggering Caspase-1 and Nlrp3 and leading to a hyperinflammatory and pyroptotic state.[43,44]

Discussion

The spike protein has gained much attention for its role in cell entry and as a target of neutralising immunity. Here, we discuss entry-adjacent roles of Spike in initiating an inflammatory 'cytokine storm', triggering barrier dysfunction, and silencing host immunity to promote and sustain infection.

Multiple experimental modalities have been used to explore the spike protein as a functional ligand, sometimes with contrasting results. These treatments are often linked to different stages of SARS-CoV-2 infection, where the spike protein can either be presented upon entry into cells, intracellularly during protein synthesis, or in sera from infected patients as its constituent subunits. The spike protein and its subunits can be present extracellularly and intracellularly during and post-infection, which may affect how a cell responds to this ligand. One study found that in patients with post-acute sequelae, spike protein antigen can persist for upwards of 12 months.[45] By contrast, the spike protein is not detected in the serum of patients 9 days following primary vaccination, and is undetectable following a secondary vaccination.

Furthermore, different subunits of the spike protein themselves are linked to specific signalling effects, but it remains unknown how each component affects signalling and whether they act independently or cooperatively. Additionally, the cellular machinery required to respond the spike protein or subunits to mediate non-entry function has not been defined. For example, ACE2 is inessential for induction of TGF-β.[28] It is therefore highly plausible that different cell types will exhibit a range of responses, and may exceed the cell population susceptible to infection.

We are clearly only beginning to uncover the mechanisms by which the spike protein can deregulate cellular signalling pathways. Here we have reviewed roles for the spike protein in addition to its primary function in mediating cell entry and how these activities may affect COVID-19 pathology. We also highlight that cells can be exposed to the spike protein through various methods and forms, and that these may have distinct disease implications.

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An update on Long COVID

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ABSTRACT

'Long COVID' is a major dilemma, difficult to diagnose and even more challenging to treat. Millions are still being affected globally and ~10% of people experience Long COVID following acute infection. Many complain about fatigue, brain fog and mental difficulties, and ~200 symptoms are described making diagnosis difficult. Both acute COVID-19 and Long COVID can cause organ damage – involving the heart, lungs, kidneys, and brain; as well as inflammation, and studies suggest that severe COVID-19 is dominated by endothelial and immunological dysfunction, and immunothrombosis. Diagnostic tests for Long COVID are largely in development and finding effective therapies for Long COVID has been a major challenge; however, it is likely that antivirals have a role in preventing and treating Long COVID. Real-world data support the effectiveness of COVID-19 vaccines in reducing the risk of Long COVID. Long COVID remains a major challenge that needs considerable on-going research to determine effective treatments. The global public health emergency may be over but the fallout of Long COVID will be with us for some time.

Keywords: antiviral drugs, coronavirus, COVID-19, Long COVID, PASC, SARS-CoV-2.

'Long COVID' is a major dilemma, difficult to diagnose and even more challenging to treat. Millions are still being affected globally by Long COVID and many thousands have succumbed in the USA alone. Australian research suggests that some 10% of COVID-19 cases are affected, based on symptoms being present 3 months after onset of acute COVID-19 (a diagnostic requirement); some suffer for years, though most cases are likely to resolve within 1 year.[1] In a recent review, it is estimated that at least 65 million individuals worldwide have Long COVID, and that ~10% of people experience Long COVID following acute infection, with 50–70% of people experiencing lasting symptoms following severe infection.[2]

Although a universal definition has yet to be determined, the World Health Organization (WHO) defines Long COVID as the continuation of symptoms, or the development of new symptoms, 3 months after the initial SARS-CoV-2 infection (COVID-19), with these symptoms lasting for at least 2 months with no other explanation. Long COVID can occur after any COVID-19 infection including subclinical infection.[3]

The Australian government Standing Committee on Health Report relating to COVID-19[4] has recommended the establishment and funding of a single COVID-19 database by our new Australian Centre for Disease Control to *inter alia*, document post COVID-19 complications (post-acute sequelae, PASC, of COVID-19) and establish a nationally co-ordinated research program involving basic science, clinical trials and implementation science. Evidence-based guidelines for diagnosis and treatment are needed and are in development.[4,5]

Long COVID can be debilitating. Many complain about fatigue, brain fog and mental difficulties, and ~200 symptoms are described. This makes diagnosis difficult. Nervous system disorders, such as postural orthostatic tachycardia syndrome (POTS) are commonly reported, often comorbidly with chronic fatigue syndrome (CFS). There are increasing reports of cognitive sequalae post COVID-19 infection, e.g. 6 months after acute symptoms have gone. Using magnetic resonance imaging (MRI), changes have been reported to the brain stem and front lobe in areas associated with fatigue, insomnia, anxiety, depression, headaches, and cognitive issues. Lasting lung damage has also been seen in children and teens with Long COVID using MRI technology.[6] Furthermore, many symptoms can be caused by another malady, making it vital to obtain a full understanding of symptoms, signs, and underlying conditions. Importantly, CFS, though separate, shares several similarities with Long COVID and often follows a viral respiratory condition.

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Severe COVID-19 disease can increase 'the risk of cardiac arrest, death, diabetes, heart failure, pulmonary embolism and stroke', as determined by analysis of the US Department of Veterans Affairs databases. Cases of severe acute COVID-19 disease, that require admission, have a higher risk of developing Long COVID. However, the majority of COVID-19 cases only have mild symptoms but can still develop Long COVID: the fact is that patients – who had mild to moderate acute illness – make up most people who go on to suffer from Long COVID.[2] In a small US study, 41% of patients with Long COVID had never tested positive for the virus except that they were found to have specific antibodies in their blood that indicated exposure to COVID-19.[7]

What risk factors predispose patients to Long COVID? Those with a history of allergies, anxiety, depression, arthritis, autoimmune diseases, nervous system disorders, chronic infections, diabetes (types 1 and 2), and obesity are more likely to be affected. There is also a higher prevalence of Long COVID in women – especially in perimenopausal and menopausal women and those under 50 years are also much more likely to develop PASC than men of similar age.[8] Research has also shown that being overweight negatively affects the body's immune response, impairing antibodies to fight the virus.[9] Having said that, being overweight does not seem to affect the immune response generated by COVID-19 vaccines. People cannot be blamed for developing Long COVID because exposure to the infection is almost impossible to prevent. Vaccination, adequate ventilation, as well as masking and other hygiene interventions are helpful but render imperfect protection.

The pathology of the virus is under intense scrutiny. Both acute COVID-19 and Long COVID can cause organ damage – including the heart, lungs, kidneys and brain – as well as inflammation, potentially leading to other issues, such as diabetes. Putative mechanisms underlying Long COVID's pathogenesis include abnormal neurological signalling, autoimmunity and immune dysregulation (including decreased production of SARS-CoV-2 antibodies), disruption of the microbiota, abnormal clotting, persistent reservoirs of virus or spike antigen, reactivation of underlying pathogens (e.g. Epstein–Barr Virus, Herpes Zoster or Shingles, and Herpes Simplex or Bell's palsy), priming of the immune system by molecular mimicry, endothelial and immunological dysfunction, and immunothrombosis.[10,11] However, studies suggest that severe COVID-19 is dominated by endothelial and immunological dysfunction, and immunothrombosis.

There is no clear understanding why SARS-CoV-2 can result in severe outcomes or why symptoms persist, whereas other human coronaviruses just cause common colds. However, one recent study, which used artificial intelligence methodology, has shown that fragments of the SARS-CoV-2 virus may drive inflammation by mimicking the action of specific immune molecules in the body.[12] It is likely that viral protein fragments, generated after the SARS-CoV-2 virus can mimic a key component of the body's machinery for amplifying immune signals (RECOVER: Researching COVID to Enhance Recovery, see https://recovercovid.org, accessed 2 March 2024).

Diagnostic tests for Long COVID are largely in development and focus predominantly on biomarkers such as proteins, hormones, endothelial/vascular biomarkers, and inflammatory monocytes to name a few.[13,14] However, results from a recent study suggest that complement biomarkers could facilitate the diagnosis of Long COVID, which also raises the possibility of using available inhibitors of complement activation to treat Long COVID.[15]

Finding effective therapies for Long COVID has been a major challenge and there is no broadly effective treatment. An important issue is that there are, almost certainly, multiple deleterious 'paths of destruction' so no one-fits-all-treatment can be applied. Specific medications (like inhaled steroids for shortness of breath), the application of cognitive strategies for brain fog, dietary changes, optimising sleep and the use of antiviral drugs may each be helpful. A few studies have shown that Paxlovid is useful to help resolve Long COVID more quickly in some cases.[16,17] More generally, in people at high risk of progression to severe COVID-19, Molnupiravir use within 5 days of SARS-CoV-2 infection may reduce the risk of Long COVID.[18] Both Paxlovid and Molnupiravir were associated with lower all-causes mortality risk compared with no antiviral use for the treatment of acute COVID-19.[19]

It is worth noting that Paxlovid is authorised for use in children as young as 12 years old but Molnupiravir isn't authorised for people younger than 18 years as it may affect bone and cartilage growth. Molnupiravir, is not recommended for pregnant individuals because animal studies suggest it could cause foetal harm. Simnotrelvir (a protease inhibitor) has also been shown to reduce symptoms of COVID-19 for those with mild infections.[20,21]

'COVID rebound' (recurrent symptoms), shortly after Paxlovid treatment was completed, has also been described, but is mild and short-lived, resolving on average in 3 days without additional antiviral treatment.[22] Paxlovid contains both Nirmatrelvir, a protease inhibitor that blocks SARS-CoV-2 from replicating, and Ritonavir, which boosts Nirmatrelvir by slowing its metabolism in the liver. However, care must be taken with the use of Paxlovid as Ritonavir can slow the metabolism of several important other drugs, thereby increasing their concentration in the blood. In some patients, drug interactions can occur but these can be managed by several means: temporarily withholding treatment, adjusting the dose or using an alternative concomitant medication. This can be time consuming the first few times the physician reviews a patient's current medications.

It is likely that antivirals have a role in preventing and treating Long COVID.[23,24] In other studies, various treatments have been effective for population subsets[2] and a variety of treatments to relieve Long COVID symptoms have been tried with mixed success, including: low-dose naltrexone,[25] antihistamines, anticoagulant regimens and apheresis.[26] Using specific monoclonal antibodies as therapy may also reduce infection risk – with the greatest benefit in immunocompromised persons including those receiving organ transplants. Such antibodies may also help immunocompromised patients with Long COVID.[27] Additionally, Coenzyme Q_{10} and D-ribose supplements have shown promise in treating Long COVID.[28] Ideally, to tackle any pandemic and its aftermath requires the availability of not only

effective vaccines but also accessible effective drugs that target excessive inflammation using inexpensive repurposed generic drugs. A recent review focusing on statins, ACE inhibitors and angiotensin receptor blockers suggests that these drugs help maintain or restore endothelial barrier integrity.[29,30] Many patients have turned to alternative medical treatments (including plasma exchange) but evidence for benefit is limited. Finally, it is worth noting that exercise use can assist mild–moderate cases but may be harmful in more severe cases (e.g. POTS), where pacing may be more effective and the input of a specialist program of rehabilitation is important.

Can Long COVID be avoided? It is best prevented by not getting COVID-19 in the first place. It is likely that by the end of 2024, more than 90% of people will have been exposed to COVID-19 at least once, but many repeatedly, so, clearly, PASC and Long COVID cases will keep on emerging. Many people have become blasé about annual vaccination even though vaccination can reduce the risk. In early studies the Australian Institute of Health and Welfare reported that, if you still catch COVID19 after two vaccination doses, there is a 13–47% lower risk of symptoms persisting beyond 4 weeks, compared to unvaccinated people who catch COVID-19.[1] Furthermore, in a recent study in Hong Kong, involving over 1 million patients, real-world data supported the effectiveness of COVID-19 vaccines in reducing the risk of post-COVID-19 long-term health consequences in patients who had a primary vaccination course or a booster dose.[31] This is encouraging and further highlights the importance of receiving booster vaccines, especially for those vulnerable to severe disease from COVID-19, but also for those not at risk and wanting some protection, albeit imperfect, against the development of Long COVID.

It is important to return to the recent development in Australia of a national plan for Long COVID, which recognises the chronic nature of Long COVID and the need for multidisciplinary team-based healthcare. The plan is focused on; strengthening primary healthcare services, improving COVID-19 vaccination communications, educational support for healthcare providers, and has a national research program involving an A$50 million investment. It also seeks to ensure all people with Long COVID and their families and carers can readily access support and treatment to achieve the best possible outcomes.[32]

Long COVID remains a major challenge that needs considerable on-going research to determine effective treatments. The global public health emergency may be over but the fallout of Long COVID will be with us for some time. Long COVID cases are increasing daily.

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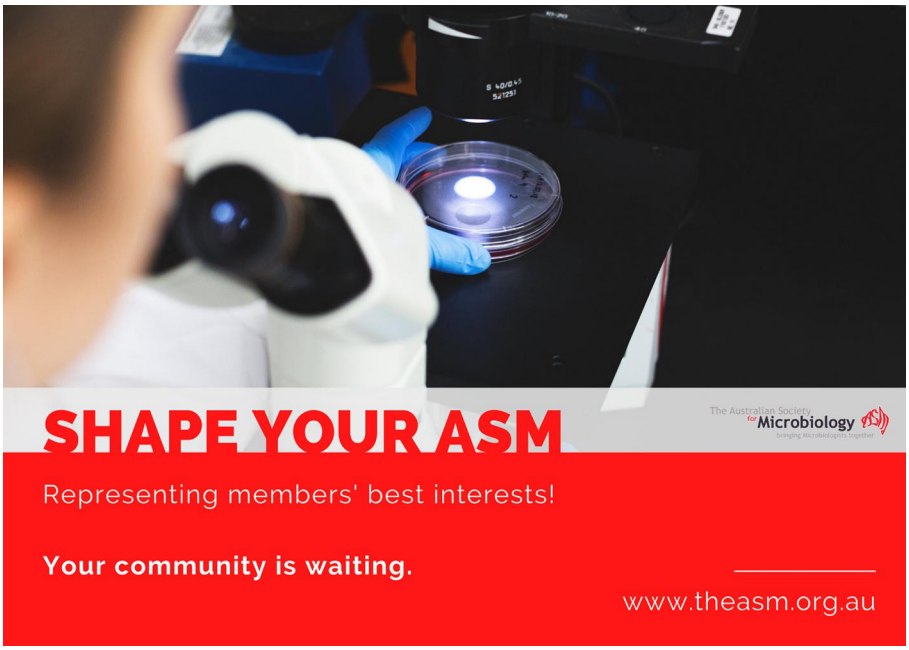

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A brief survey of interventional agents intended to treat Long COVID

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ABSTRACT

The present study provides a brief survey, based on a search of the US National Institutes of Health dataset Clinicaltrials.gov, of clinical trials for interventions that could prevent, mitigate or cure Long COVID, a syndrome of increasing concern to patients and their physicians, as the acute phase years of the main pandemic recede and some patients remain afflicted by the failure of the disease signs to completely abate. The disease is pleomorphic in its presentations and severity, with the consequence that there is no one generally accepted approach to treatment, and clinical trial design can be a challenge. At time of writing, there is no approved therapeutic intervention or combination of interventions for Long COVID. Over the last 3 years, there have been several reviews of the state-of-play in relation to therapies for long COVID; however, this is a rapidly moving field and the intention of this brief article is to provide a succinct update on a subset of potential interventional therapies that are currently undergoing clinical trial. There are at least 82 unique active agents in development, and they are characterised by diverse mechanisms of action; however, the emergency approach that was employed during the COVID-19 pandemic is not being replicated for development of treatments for Long COVID.

Keywords: COVID, post-acute COVID-19 syndrome, post-acute sequelae of SARS-CoV-2, post-acute sequelae of COVID-19, long haul COVID, persistent COVID-19, post-acute COVID syndrome, long hauler COVID, chronic COVID syndrome.

Introduction: Long COVID

Some individuals who have been infected with SARS-CoV-2 (COVID-19) experience long-term effects from their infection, lasting many months or even years, and characterised by a broad range of sequelae affecting pulmonary and extrapulmonary organ systems.[1] This syndrome is known as 'Long COVID' or Post-COVID Conditions (PCC).[1] Long COVID is broadly defined as signs and symptoms that continue or develop after acute COVID-19 infection (definition provided by the US Department of Health and Human Services, DHHS, and Centers for Disease Control and Prevention, CDC[2]).

- Long COVID occurs more often in people who had severe COVID-19 illness, but anyone who has been infected with the virus that causes COVID-19 can experience it.
- People can be reinfected with SARS-CoV-2, the virus that causes COVID-19, multiple times. Each time, they have a risk of developing Long COVID.

Most people with COVID-19 improve to their baseline health within a few days to a few weeks after infection; at least 4 weeks after infection is the time after which Long COVID would normally be first identified. Healthcare providers consider a diagnosis of Long COVID based on each patient's health history, including a diagnosis of COVID-19, either by a positive test or by symptoms or exposure, as well as based on a health examination.

For some people, Long COVID can last weeks, months or years after COVID-19 illness and can sometimes result in long-term disability. Sometimes the symptoms disappear and return. The condition is extremely pleomorphic across the assessed cohort. Some individuals with Long COVID have symptoms that are neither explained by tests nor easy to manage.

Some people may be more at risk for developing Long COVID:

- People who have experienced more severe COVID-19 illness, especially those who were hospitalised or needed intensive care.

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- People who had underlying health conditions prior to COVID-19.
- People who did not receive a COVID-19 vaccine.
- Individuals who had repeated COVID-19 infections.

The pleomorphic nature of disease, and concomitant definitional issues for Long COVID, could make clinical development challenging, given the requirement for definition of consistent endpoints for trials. The present study was conducted to provide a brief survey, based on a search of the US National Institutes of Health dataset known as Clinicaltrials.gov (see https://clinicaltrials.gov/, accessed 26 December 2023), of progress in conducting clinical trials for interventions that could prevent, mitigate, or cure Long COVID, a syndrome of broad and increasing concern to previous COVID-19 patients and their treating physicians as the acute phase years of the main pandemic fade and some patients remain afflicted by the failure to completely abate of the disease signs. Moreover, the continuing bursts of COVID-19 worldwide have also led to a new cohort of patients with COVID-19 acute phase disease; thus, the number of Long COVID patients continues to increase.

The known current treatment options for Long COVID generally consist of symptomatic treatment and experimental therapies, the latter of which are captured in the present paper, to the extent possible within the limitations of the accessible database. Symptomatic treatment varies widely, since the disease is quite pleomorphic in its presentations and severity, with the consequence that there is no one generally accepted treatment methodology. At time of writing, there is no approved therapeutic intervention or combination of interventions for Long COVID.

Over the last 3 years, there have been several reviews of the state-of-play in relation to therapies for Long COVID (e.g. Chakraborty and Bhattacharya,[3] Bramante *et al.*,[4] Novak,[5] Ceban *et al.*,[6] Chee *et al.*[7]); however, this is a rapidly moving field and the intention of this brief article is to provide a succinct update on a subset of potential therapies that are currently undergoing clinical trial.

Methods

Using the term 'Long COVID', and the synonyms 'Post-acute COVID-19 syndrome', 'Post-acute sequelae of SARS-CoV-2 infection', 'Post-Acute Sequelae of COVID-19', 'long haul COVID', 'persistent COVID-19', 'post-acute COVID syndrome', 'long hauler COVID' and 'chronic COVID syndrome', the authors of this paper searched the entire database of the US government's Clinicaltrials.gov (see https://clinicaltrials.gov/) to assess all clinical trials under US Investigational New Drug Applications (INDs) related to Long COVID, both interventional and observational, and in Phases 1–4 of clinical trial study. The results were then identified as interventional or not (interventional was the only term used, and only drug, biological and medical device trials, as reviewed for each of the 500 hits from the search, were further explored, see below). The interventional trials were further classified as to whether the experimental agent was: a small molecule drug; a biological drug, including vaccine; a medical device; or dietary supplement or traditional medicine. Please note that we used a narrower definition of 'interventional' than has been used previously for the purpose of such a survey.[3] There are many behavioural and other approaches used as interventions for this constellation of conditions. We decided to concentrate on small molecule drugs, biologic drugs or vaccines, and medical devices. The classification of trials as drug, biological or medical device was by author discretion based on information available from the database and was a straightforward determination, even when precise chemical structures were not disclosed. No trials were excluded if they fit the criteria for inclusion, and actives were identified in almost all cases.

The authors are most familiar with these interventions and have a long history in those areas and we believe that it is reasonable to assert that these types of intervention are the most likely to receive broad global acceptability for Long COVID. Regulatory approval of these interventions is comparatively straightforward, but safety and efficacy must be proven prior to broad use of such interventions. Psychotherapeutic, behavioural, and similar controlled or uncontrolled approaches were not covered in our concept. Thus, the number of discrete interventions is quite small as delineated here. It is important to note that we were unable to categorise some agents because proprietary information from INDs cannot be released by the government and some of the experimental agents were only identified by code number.

A search on 'Long COVID' (and synonyms as delineated above) in all phases of clinical trials and received exactly 500 'hits'. We then manually went through every citation and exempted from the list the types of interventions that did not fit the categories described above.

A further screen for Phase 3 studies was made to attempt to identify those interventional agents under experimental use for Long COVID that were furthest along the developmental pathway for treatment of the condition. It should be understood that, as above, proprietary IND information cannot be provided to the public by the US government, and the actual regulatory status of an interventional agent in a clinical trial (e.g. whether a marketing application is imminent for a particular intervention intended for treatment of Long COVID) cannot be ascertained by such searches. Further, the search results were rescreened using new discriminators on the original search terms to include only Phase 3 and larger Phase (e.g. 4) trials. The results represented the small number of interventions ($n = 26$) that were farthest along in the clinical trial process. These are either interventions previously approved or cleared for other indications and being tested for Long COVID, or interventions which have moved quickly through the process.

Results

Table 1 provides a delineation of interventional agents intended to treat Long COVID from the clinical trial database, shown by type of intervention, number of separately identifiable active interventions of each chosen type, and any comments needed to further refine these results. It can

Table 1. Number and type of interventions (https://www.clinicaltrials.gov/, accessed November 2023).

Type of intervention	Number of actives	Number of trials	Comments
Small molecule drug	31	40	There are more than 31 trials due to multiples for a small number of interventions. Nirvitelvir or Ritonvir: 4 trials
			Naltrexone: 3 trials
			Fluvoxamine: 2 trials
			Remdesivir: 2 trials
			Methylprednisone: 2 trials
			Bupivacaine: 2 trials
Biological drug/stem cells	17	20	Mesenchymal umbilical stem cells: 2 trials
			Efgartigimod (humanised antibody Fc): 2 trials
			NT-17 (Long-acting Interleukin-7): 2 trials
Medical device	18	26	There are more than 18 trials due to multiples for a small number of interventions
			Vagus nerve stimulation: 2 trials
			Hyperbaric oxygen: 2 trials
			HD-DCS (High Definition Transcranial Direct Current Stimulation or DCS: 6 trials
			Immunoadsorption with Therasorb column: 2 trials
Dietary supplement/traditional medicine	29	30	Probiotic supplement: 2 trials
Unclassified	5	5	Unable to identify the active principle, generally suspected traditional medicines or dietary supplements

be seen that a large number (78%) of the 500 clinical trials extracted from the database were noninterventional; these were not considered in further analyses of the data. Most of the trials were in early clinical phases (Phase 1 and 2); at the time of search, only a small number (26) had progressed to or commenced Phase 3 or beyond. This often does not reflect potential success in approval and marketing; rather, well-known or previously approved interventions with data for a new indication may be allowed to progress into more advanced clinical trials more quickly for indications with unmet medical need and, often, greater severity (e.g. Long COVID). Table 1 provides specific numbers of discrete, identified (or unclassified) types of interventions across the *interventional* results from the database. It is noteworthy that a number of trials have been, or are being, conducted with the same agent; this is due either to different investigators or sponsors pursuing the same intervention, or multiple trials conducted by the same sponsor using the same intervention. The number of trials, retrieved from the database, that were classified as interventional was 121 (see Table 1).

A majority of the trials involve small molecules (33%) and, in descending order, dietary supplements or alternative medicines (27%), medical devices (17%) and biologic drugs (14%).

Table 2 presents a discrete subset of the data involving only interventional Phase 3 (advanced or confirmatory) clinical trials on specific agents (where identification as to small molecule, biological drug, etc. can be made directly from the available dataset, based on the authors' expertise). In interventional drug development, these interventions are typically the farthest along towards the possibility of approval by the regulatory authorities, since they have already shown some promise in earlier clinical trials.

Based on the information extracted from the database search, the following agents are most likely to move into the final phase of development and potential marketing (see Table 3).

These interventions were selected for Table 3 either because they are furthest along in the clinical trial confirmatory process; they have shown the most promise in previous clinical studies, or they have been previously approved, which renders approval or licensing more likely for new indications, given evidence of statistically significant and clinically relevant efficacy and acceptable safety in the patient cohorts selected in the Long COVID environment.

Conclusion

The pleomorphic nature of disease, and the consequential definitional issues for Long COVID, present a challenge for clinical development, given the requirement for definition of consistent endpoints for trials. Of the discrete interventional agents appearing in the clinical trials database, only ~1/3 are in Phase 3 clinical trials at present. Based on the number of patients enrolled, some of these listings are almost certainly for Phase 2 or 3 clinical trials, not typical Phase 3 confirmatory trials. Although there are at least 82 unique active agents (including dietary supplements, but excluding medical devices) in development, and they are characterised by diverse mechanisms of action, the emergency approach that was employed during the COVID-19 pandemic is not being replicated for development of

Table 2. Active principles in Long COVID Phase 3 clinical trials.

Active principal	Type of intervention	Combination of active ingredients/interventions?
Metformin	Small molecule	No
Lau-7B	Small molecule	No
Nitrite Supplementation	Dietary supplement	No
Fluvoxamine	Small molecule	No
Na Pyruvate Nasal Spray	Small molecule	No
Sirulimus C-19	Small molecule	No
Pycnogenol	Dietary supplement	No
Trigeminal Nerve Stimulation	Medical device	No
Testofen	Dietary supplement	No
Anakinra	Small molecule	No
Human Growth Hormone	Biologic	No
Homeopathic Treatment	Dietary supplement or alternative	Not discernable from database
Statins	Small molecule	Not discernable from database
Prospekta	Biologic	No
Allopurinol	Small molecule	No
Lidocaine Stellate Ganglion Block	Small molecule	No
Paxlovid	Small molecule	Yes
Immunorecon	Dietary supplement	Not discernable from database
Adaptogens	Dietary supplement	Yes
ASA	Small molecule	No
Montelukast	Small molecule	No
COVID-19 Vaccines	Biologic	Yes

Table 3. Lead interventions for Long COVID that appear to be farthest progressed toward market.

Intervention	Structure	Comments
LAU-7B (Sponsor: Laurent Pharma)	Retinoid	Anti-inflammatory Phase 2 and 3 Adaptive Trial
Metformin (Sponsor: University of Minnesota)	Small molecule	Recipients of metformin were 41% less likely to develop Long COVID.[4] Antiviral, antidiabetic, anti-inflammatory actions
Anakinra (Sponsor: Hellenic Institute for the Study of Sepsis)	Biologic, recombinant Il-1 receptor agonist	Approved for treatment of Rheumatoid Arthritis
Statins (Sponsor: The George Institute, Sydney Australia)	Small molecule. Fermentation product of aspergillus, HMG-COA reductase activity	Approved for lipid reduction and other indications
Paxlovid (Sponsor: Kanecia Obie Zimmerman, Duke Universityand Stanford University).	Small molecules (2)	Combination of two antivirals, Nirmatrelvir co-packaged with Ritonavir, previously approved for moderate to severe acute COVID-19
Montelukast (Sponsor: Fundacio d'Investigacio en Atencio Primaria Jordi Gol i Gurina)	Small molecule	Leukotriene receptor antagonist, previously approved for asthma.

treatments for Long COVID. Thus there is likely to be significant delay before medical professionals will be able to offer patients commercially available treatments for Long COVID, unless there is a redirection of government or private funding.

We look forward to following up this study with further assessments of the development of interventions for mitigating or curing Long COVID in the months and years to come.

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Data availability. Outputs from the search based on the search terms listed in the methods section can be provided in PDF format, on request.

Conflicts of interest. Ross Barnard is a member of the editorial board of *Microbiology Australia* but did not at any stage have editor-level access to this manuscript while in peer review. *Microbiology Australia* encourages its editors and editorial board members to publish in the journal and they are kept totally separate from the decision-making processes for their manuscripts. The authors have no further conflicts of interest to declare.

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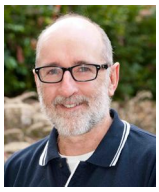

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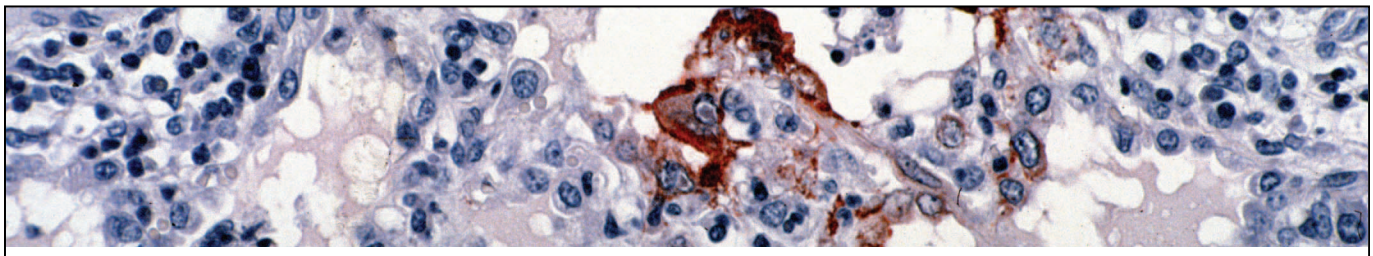

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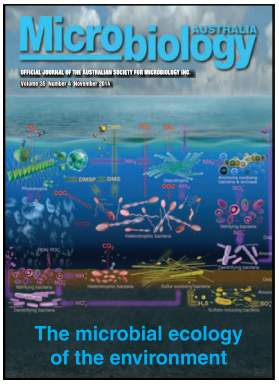

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Australia's COVID-19 vaccine journey: progress and future perspectives

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ABSTRACT

COVID-19 vaccines have played a pivotal role in reducing SARS-CoV-2 disease severity and mortality. However, evolutionary pressure has resulted in viral variants with increased fitness, greater capacity for immune evasion and higher infectivity. This evolution is exemplified by the emergence of the Omicron subvariants, all of which demonstrate significant escape from vaccine- or infection-induced immunity. Broadly protective vaccines are urgently needed to fight current, emerging and future SARS-CoV-2 variants. Australia is actively contributing to these efforts through the development of innovative vaccination approaches and vaccine delivery platforms.

Introduction

The strict public health measures implemented across Australia during the early phase of the COVID-19 pandemic kept rates of SARS-CoV-2 infection extremely low, until most of the population had received at least two doses of an approved vaccine. Since the relaxation of these measures in late 2021, a combination of waning immunity post-vaccination and the highly infectious and immunoevasive nature of the Omicron variants has driven waves of infection. The initial emergence of Omicron was characterised by the dominance of a single subvariant, BA.1, which was rapidly replaced by BA.2 and then BA.5 by mid-2022[1] (Fig. 1*a*). Subsequently, infection waves in Australia have consisted of a diverse mix of Omicron subvariants that has been termed the Omicron 'soup' (Fig. 1*a*). Thus, immunity to SARS-CoV-2 across the Australian population is highly variable, depending on both the number of vaccine doses received or breakthrough infections.

The COVID-19 vaccine rollout in Australia and its effect on population immunity

Over 95% of adults in Australia had received the initial two doses of an approved COVID-19 vaccine by the end of 2021. Over time, the levels of SARS-CoV-2 neutralising antibodies (NAbs) naturally decline. This, coupled with the emergence of viral variants carrying mutations that diminish antibody recognition, contributes to a reduction in vaccine effectiveness. Australian researchers were the first to establish a correlation between the efficacy of COVID-19 vaccines and the titres of NAbs targeting the SARS-CoV-2 spike protein.[2–4] To counter waning immunity, 'booster' vaccine doses are recommended by the Australian Technical Advisory Group on Immunisation (ATAGI); 3rd vaccine doses were initially introduced at the end of 2021 for high-risk groups, before being expanded in 2022 to all adults whose second dose had been received more than 6 months prior. Clinical data from the Australian population have shown that such booster vaccine doses can restore NAb levels after waning, broaden cross-recognition of SARS-CoV-2 variants and improve protection against symptomatic infection and severe disease.[5] Importantly, Australia served as a distinctive case for evaluating booster efficacy, as prior to Omicron, the Australian population had low rates of previous SARS-CoV-2 infection but high levels of vaccination. In older Australians (65+ years), vaccination is highly effective against COVID-19 mortality, although effectiveness wanes quickly with time since last dose.[6] Accordingly, ATAGI recommends an additional booster dose for those 75+ years, if their previous vaccination was more than 6 months prior.[7] However, uptake of booster doses has been slow; as of 6 December 2023, only

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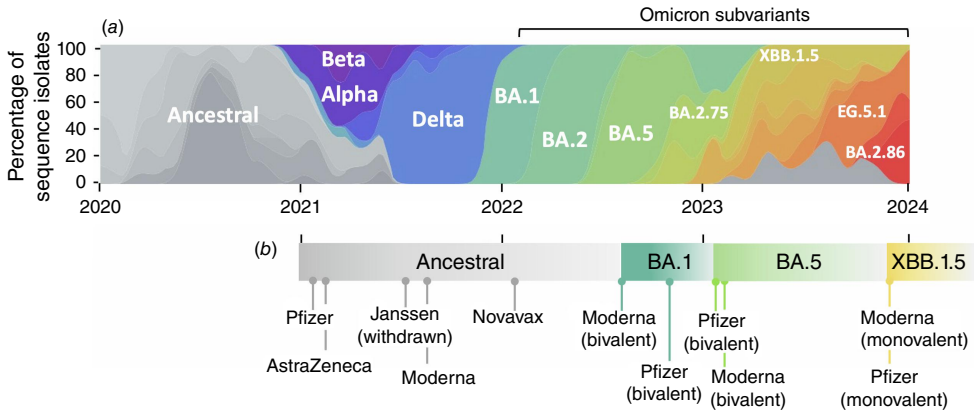

Fig. 1. SARS-CoV-2 variants and vaccination in Australia. (*a*) Overview of SARS-CoV-2 variants circulating in Australia over time as percentage of total infections. Only representative variants shown for clarity. Data from CoVariants.org. (*b*) Timing of the vaccine rollout in Australia. The date of provisional approval for each vaccine (by company and variant) is approximated on the timeline. Full details can be found at www.tga.gov.au/products/covid-19/covid-19-vaccines/covid-19-vaccines-regulatory-status.

22% of those aged 75+ years in Australia have received a COVID-19 booster within the last 6 months, leaving a large number of vulnerable individuals at risk.[7]

Currently, population immunity to SARS-CoV-2 in Australia is being shaped by a mix of responses to both vaccination and infection,[8] largely with the Omicron subvariants (Fig. 1*a*). The ability of Omicron subvariants to cause breakthrough and re-infections can be attributed to key mutations within the viral spike protein that render them antigenically distinct from the ancestral spike antigen used in the original COVID-19 vaccines. Therefore, to improve responses against these subvariants updated bivalent (ancestral + BA.1 and ancestral + BA.5) booster vaccines were developed (Fig. 1*b*). Bivalent booster vaccines were provisionally approved in Australia in August 2022, and were recommended by ATAGI as the booster of choice in early 2023. A study of Australians aged over 65 showed bivalent boosters provide better protection against mortality from the diverse Omicron subvariants XBB and BA.2.75 than ancestral-based vaccines.[9] Owing to the persistent global dominance of the Omicron subvariants, particularly the recombinant XBB lineage, monovalent XBB.1.5 booster vaccines were developed and approved for use in Australia in October 2023. Currently there are limited studies describing the efficacy of XBB.1.5 monovalent vaccines; however, current data suggest the XBB lineage, which includes EG.5 variants, could be soon replaced by JN.1 variants.[10] Therefore, the development of next-generation COVID-19 vaccines that can provide pan-variant protection, as well as more durable responses, are required to reduce SARS-CoV-2 infection rates.

Australia's contribution to the next-generation of SARS-CoV-2 vaccines

Australia's dependence on 'imported' COVID-19 vaccines significantly influenced the pace of the initial vaccine rollout, highlighting vulnerabilities in our sovereign vaccine manufacturing capabilities.[11] Nevertheless, the pandemic has catalysed increased investment, both domestically and globally, in advancing not only COVID-19 vaccines but also tools to combat diseases with pandemic potential. This investment, coupled with the high quality of Australian science, has given rise to several research programs addressing these issues, as outlined in Table 1 and discussed below.

Improved variant-based vaccines

The emergence of the SARS-CoV-2 Beta variant in late 2020, the first variant of concern (VOC) to display significant immune evasion, initiated the development of variant-based vaccines that could induce cross-protection. We showed that in animal models, booster vaccines based on the Beta variant could broaden immunity against divergent VOCs.[12] Researchers at The University of Melbourne and Monash Institute of Pharmacological Sciences (MIPS) developed two Beta-based vaccine candidates (protein and mRNA respectively) that showed promise in Phase I clinical testing[13] (Table 1). In individuals who had already received three COVID-19 vaccine doses, both candidates were able to boost antibody responses, including against the highly immune-evasive Omicron subvariants XBB.1.5 and BQ.1.1.[13]

Vaccines targeted to circulating variants have been the basis for all authorised COVID-19 booster shots. However, the virus has demonstrated the ability to surpass the pace of vaccine development and distribution, as illustrated in Fig. 1*b*. Consequently, significant attention has been directed towards creating broadly protective, pan-variant candidates to 'futureproof' against both existing and emerging SARS-CoV-2 variants. Supporting these programs has been a major focus of the Coalition for Epidemic Preparedness Innovations (CEPI), a global partnership dedicated to accelerating the development of vaccines against epidemic and pandemic threats. With the support of CEPI, and in collaboration with Bharat Biotech International and ExcellGene SA, we have developed chimeric spike antigens (CSAs) that display broad immunological cross-reactivity and high-yield

Table 1. Overview of COVID-19 vaccine development in Australia.

Lead organisation	Technology	Status	Reference
Improved variant-based vaccines			
The University of Melbourne	Beta variant receptor binding domain (RBD) in MF59 adjuvant	Phase I complete	13
Monash Institute of Pharmacological Sciences	Beta RBD mRNA vaccine	Phase I complete	13
The University of Sydney	Pan-variant, chimeric spike protein in SWE adjuvant	Pre-clinical, Phase I scheduled for Q3 2024	14
Garvan Institute of Medical Research	Pan-variant mRNA	Pre-clinical	15
New vaccine formulations and delivery approaches			
Vaxine	Spike protein in Advax adjuvant	Approved for use in Iran	16
Vaxxas	Spike protein and QS21 adjuvant coated skin nanopatch	Phase I ongoing	20
University of Sydney	DNA vaccine. Needle-free injection system	Phase I ongoing	22
University of Adelaide	DNA vaccine. Needle-free injection system	Phase I ongoing	23
University of Sydney	Spike protein in Advax adjuvant. Mucosal delivery	Pre-clinical	17
Centenary Institute	Spike protein with Pam2Cys adjuvant. Nose-only mucosal delivery	Pre-clinical	18
Griffith University	Codon de-optimised, live attenuated SARS-CoV-2. Mucosal delivery	Pre-clinical	19
Novel vaccine platforms			
The University of Queensland	S-CLAMP spike protein and MF59 adjuvant	Second generation CLAMP2 phase I for proof-of-concept complete	24
EnGeneIC	Nanocell packaged with spike protein plus a-galactosyl ceramide adjuvant	Phase I/IIa ongoing	27
Sementis	Non-replicating vaccinia virus vector encoding spike protein	Pre-clinical	26

Details of the listed vaccines were obtained by examining published information, including journal articles and press releases. The list is representative and may not include all candidates currently in development.

manufacturability.[14] CSAs are designed to incorporate mutations known or proposed to affect immunity that are present across VOCs, or predicted to arrive in future variants. An alternative approach is to develop universal COVID-19 vaccines by targeting regions of the virus that are more conserved than the spike protein; this is being pursued by a consortium of the Garvan Institute for Medical Research, Kirby Institute (at the University of New South Wales, UNSW) and the UNSW RNA Institute, with support from the NSW Health COVID-19 Vaccine Acceleration Research Grant scheme.[15]

New vaccine formulations and delivery approaches

A major challenge for control of COVID-19 is to develop strategies that can prevent viral transmission and curb the number of infections. In collaboration with the Australian biotech company Vaxine, we developed a mucosal vaccine combining spike antigen adjuvanted with the novel polysaccharide adjuvant Advax, which is a component of Vaxine's SpikoGen COVID-19 vaccine that is approved for use in Iran.[16] Our mucosal vaccine provided sustained generation of NAbs and lung resident T cells, which was not observed with parenteral immunisation, coupled with sterilising immunity against virulent SARS-CoV-2 infection in mice.[17] Additionally, Ashhurst *et al.* demonstrated that intranasal administration of spike antigen with the TLR2-stimulating adjuvant Pam2Cys stimulated anti-spike immunoglobulin A (IgA) production, generated systemic NAbs and protected K18-hACE2 mice from clinical disease and lung viral infection.[18] Progression of this vaccine is supported by the NSW Health COVID-19 Vaccine Acceleration Research Grant scheme. Mucosal delivery of a codon de-optimised, live-attenuated SARS-CoV-2 vaccine is also being explored.[19]

Needle-free delivery platforms are also being investigated to improve vaccine performance and safety. Vaxxas has developed a high-density skin microarray patch (HD-MAP), that when coated with spike protein and the QS21 saponin adjuvant, demonstrated enhanced immunity compared to intradermal delivery and provided complete protection from SARS-CoV-2 challenge in mice.[20] This vaccine has advanced to phase I clinical testing in Australia, and the technology is also being applied to mRNA vaccine delivery.[21] Needle free injection methods are also being tested locally for the delivery of DNA-based COVID-19 vaccines[22,23] (Table 1).

Novel vaccine platforms

The COVID-19 pandemic underscored the urgent need for rapid and equitable vaccine distribution, prompting the development of adaptable vaccine platforms that can be quickly updated in response to emerging threats. The University of Queensland, supported in part by CEPI, has developed a rapid response vaccine platform based on their 'molecular-clamp' technology for antigen stabilisation and manufacture. Initially deployed to create a spike protein-in-adjuvant-based COVID-19 vaccine in 2020, the first candidate faced challenges with the clamp design, limiting progression beyond phase I of clinical testing.[24] Subsequently, clinical assessment of a second-generation clamp has demonstrated viability of the technology as a versatile vaccine platform.[25] Sementis, in collaboration with the University of South Australia, have applied a replication-deficient vaccinia virus platform for SARS-CoV-2 spike protein delivery, with strong immunity observed in preclinical assessment using murine models.[26] EnGeneIC, a Sydney-based company, utilised its bacterial-derived nanocell technology to encapsulate bacterially expressed SARS-CoV-2 spike protein and an α-galactosyl ceramide adjuvant.[27] A phase I/IIa trial of this candidate is currently in progress.

Conclusions

Australia made many valuable scientific contributions during the COVID-19 pandemic that have strengthened our vaccine development and manufacturing capabilities. Continued research and investment in this field will be critical to ensure we are prepared for future pandemic threats.

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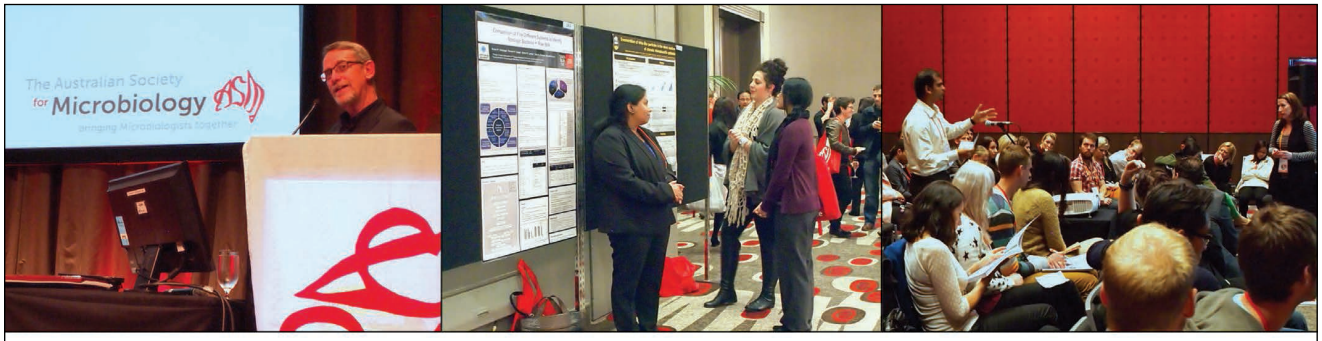

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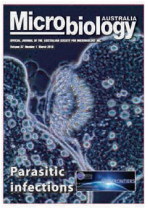

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Emerging viral threats in Australia

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ABSTRACT

Pathogenic viruses pose significant threats to human health. Consequently, it is important to consider the mechanisms by which viruses might emerge and spread both within Australia and internationally. Australia is relatively isolated from major global population centres, reachable only by international flight or long boat journey (with the exception of the most southern and eastern parts of Indonesia). This isolation, coupled with the island nature of Australia, allows broadly effective interventions to be put in place to minimise the effect of viruses circulating internationally. However, the threats posed by virus transmission emerging from within Australia, including from novel animal reservoirs as a consequence of anthropogenic activities, warrant investigation. Here we discuss the current emerging viral threats to Australia and the likelihood of a virus emerging from a domestic reservoir. We also discuss the importance of virus discovery methods for understanding the diversity and ecology of viruses in our invasive and native wildlife populations.

Keywords: animal reservoir, infectious disease, virus discovery, virus emergence, zoonosis.

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Introduction

Theoretical and technological advancements since the mid-1990s have greatly improved the ability of global public health bodies to respond to emerging pandemic threats.[1] Nevertheless, the emergence of SARS-CoV-2 and subsequent global spread of the virus during the COVID-19 pandemic has highlighted how vulnerable we remain to pandemic events in the 21st Century. Our world is rapidly changing, with an unprecedented degree of global interconnectedness, and the looming threat of diseases emerging because of anthropogenic activities and their flow on effects.[2] In the past decades, increasingly frequent extreme-weather events associated with climate change have often led to outbreaks of disease in their wake.[3] Other hallmarks of human life in the 21st Century such as urbanisation, industrialised farming and changing land use have unmistakable effects on the ecosystems around human populations, and these equally affect the dynamics of virus evolution and emergence.[2] However, not all countries are equally at risk. The likelihood of the emergence and sustained transmission of a pathogenic virus within a given region is affected by the interplay between factors such as demographics, geography, infrastructure and biodiversity. In this paper, we consider the intersection of these components to evaluate how viral threats emerge, and what the emerging viral threats to Australia might be.

Risk factors for virus introduction and transmission

Many factors contribute to the emergence of a virus in the human population, with both ecological and sociological elements being equally important.[2] The majority of emerging infections identified from the mid-20th Century have been of zoonotic origin, whereby transmission occurs from an animal reservoir into humans followed by sustained human-to-human transmission.[4] It is important to identify animals acting as reservoirs for viruses with pathogenic potential. For example, live-animal 'wet' markets have previously been identified as areas of strong concern for zoonotic pathogen spillover given that they represent an intersection between 'exotic' animals harbouring potentially novel pathogens and (often) a densely populated area with an immunologically naive population.[5,6]

The likelihood of the global spread of emerging viruses into naïve populations has increased through a high degree of global interconnectedness.[2] Australia is in a unique

position, being the most isolated inhabited continent in the world and reachable only by international flight or extensive journey by boat from major global population centres beyond Indonesia. These factors allow the risk of the spread of an introduced pathogenic virus to be reduced. For example, during the early stages of the COVID-19 pandemic, Australia's isolation enabled the rapid closure of international borders. Coupled with Australia's low-density population, this isolation from the global community enabled effective isolation of positive cases and prevented uncontrolled spread of infections across the country until the eventual relaxation of public health measures. Consequently, the trajectory of SARS-CoV-2 infections in Australia was far different from most other countries that did not have the same geographic isolation and population dynamics.[7,8]

The increasing human population demands greater resources and puts ecosystems under increasing strain through urbanisation and increased population density, which increases the risk of disease transmission.[2] Associated habitat destruction destabilises ecosystems and hastens the rate of species extinctions. We cannot predict fully how these significant ecosystem disruptions will affect virus evolution. Viral lineages could go extinct with their hosts, or, alternatively, they could adapt to increasing evolutionary pressure and become more generalist.[9,10] Industrialised farming to feed a growing population creates a dire risk for virus emergence. Genetic diversity among farm animals is often low, and the animals can be in states of poor health and high stress while in extremely dense populations. These factors are ideal for viral evolution during sustained animal-to-animal transmission. Particularly concerning examples include fur farms and farming of non-domestic species, where a number of novel and previously described viruses of concern have been identified across multiple species including outbreaks of SARS-CoV-2 and influenza in European fur farms.[5,11–13] The net result of prolonged viral evolution in response to selective pressures or prolonged transmission is an increased risk of disease emergence in the human population by zoonosis.

Anthropogenic climate change has increased the frequency and severity of extreme weather events. The aftermath of these events creates an opportunity for disease emergence as infrastructure is often damaged, and the dynamics of wildlife and disease vectors shift.[14,15] Of particular risk are events that lead to a lack of clean water and, in some cases, an increase in stagnant water around urban settlements, which can drive increased mosquito populations.[16,17] These conditions have been witnessed in Australia over the 2020–2023 period, with increased severe flooding events coinciding with both an increase in mosquito populations and an increase in arboviral disease, including the emergence of Japanese encephalitis virus (JEV) in south-eastern Australia.[18] The outbreak likely stemmed from the introduction of the virus from the Torres Strait Islands, Papua New guinea and Indonesian territories, where the outbreak genotype (IV) had previously been identified.[19] A serosurvey within this period indicated that 1 in 11 people sampled across the New South Wales and Victorian border showed evidence of exposure to JEV.[20] The JEV outbreak demonstrated the role that anthropogenic climate change can play in the broader chain of arbovirus transmission: the virus was most likely imported into Australia through infected migratory birds opportunistically seeking flooded areas, followed by spread into porcine amplifying hosts by the increased population of mosquito vectors.[19]

Despite the advantages of being isolated with a generally low population density, it is crucial that Australia is prepared for the next international pandemic event. Some key steps will include monitoring any emerging disease outbreaks globally and being prepared to act accordingly, and heeding warnings surrounding the risks associated with increased urbanisation and industrialisation.[21] However, of arguably the most importance is that we understand little about the ecology and epidemiology of zoonotic viruses in Australia and the associated risk of disease emergence.

The threat of animal reservoirs in Australia

Viruses that can jump hosts and sustain transmission in these new hosts are referred to as generalist viruses. Generalists are the most likely viruses to gain the ability to infect humans and sustain transmission within the human population through frequent exposure events, likely occurring at the human–animal interface through activities such as farming and hunting. For example, SARS-CoV-2 has jumped from wildlife into humans and subsequently spilled back into several wild and domestic animal species.[9,22] These viruses generally accumulate mutations as they adapt to their hosts that can lead to increased disease severity and transmissibility.[23] To realise fully the risk of generalist viruses emerging within Australia, it is important to consider the species richness within the continent.

Australia has a unique assemblage of endemic flora and fauna that have evolved over many millions of years in response to both reproductive isolation and changes in climate over time.[24] The incidence of emerging zoonotic diseases has a strong correlation with increased mammalian species richness.[25] This is not surprising in an evolutionary context: the phylogenetic distance effect is theoretically and empirically known to affect the success of a pathogenic host switch, with a horizontal host switch more likely to occur across more closely related species.[26,27] However, the threat is not restricted to mammals: zoonotic transmission is known to occur from other animal groups such as birds.[28] To date, several animal groups have been identified as potential and realised sources of zoonotic disease emergence in Australia. Bats, rodents and birds are established reservoirs of viruses capable of spill over into humans such as influenza, hantaviruses, lyssaviruses and Hendra virus. Sporadic Hendra virus outbreaks have occurred at the interface of domestic species and wildlife in New South Wales and Queensland since 1994, characterised by high levels of morbidity and mortality in horses and humans.[29] An outbreak of lymphocytic choriomeningitis virus was detected in New South Wales as a result of the 2021 'mouse plague' experienced in the region, and it is now suspected that the virus may be widespread in the Australian rodent population, although surveillance has not yet been conducted to

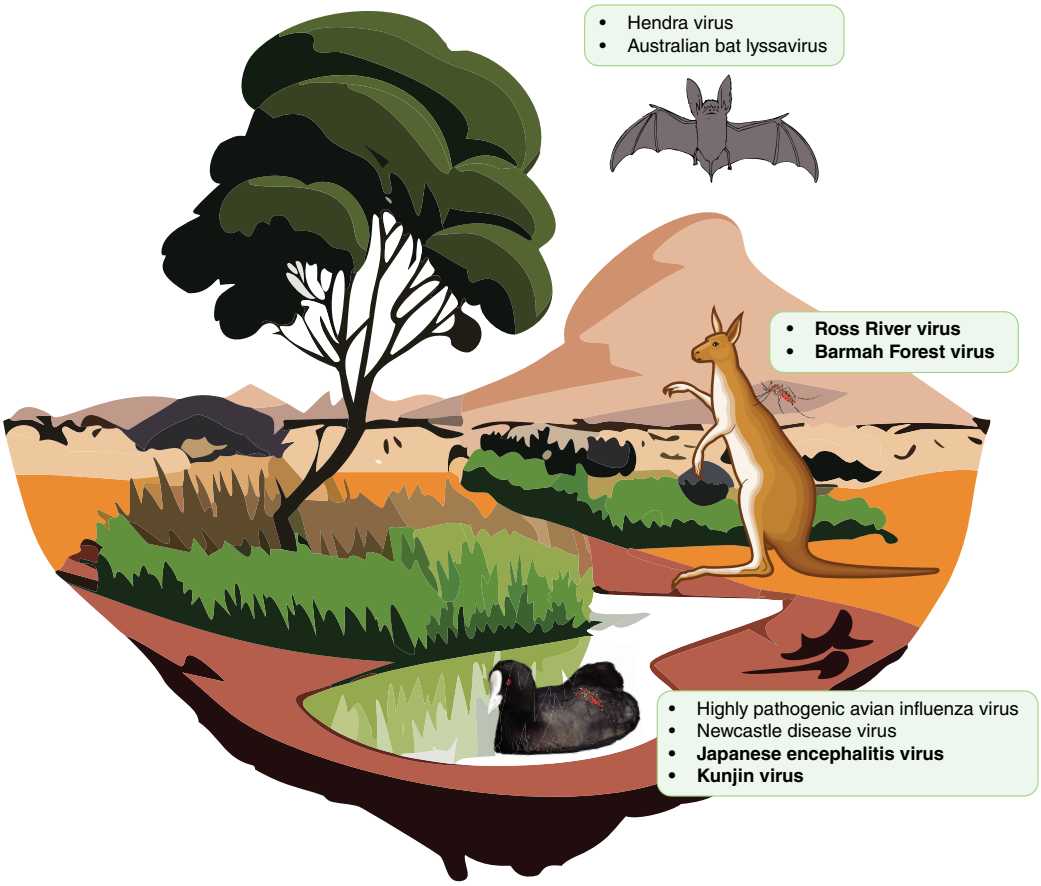

Fig. 1. Native reservoirs of zoonotic viruses in Australia, including bats, macropods and water birds, and the zoonotic viruses they are associated with. Viruses written in bold text are arboviruses vectored by mosquitoes.

determine the geographic range of the virus.[30] Macropods are suspected to be amplifying hosts for several arbovirus species (the informal grouping for any virus spread by an arthropod vector) in Australia (Fig. 1).[31–33] Introduced pigs and chickens are also known reservoirs of influenza virus.[34,35] However, there is still much to learn about the diversity and ecology of viruses in Australian wildlife, including their true range and prevalence.

To date, metagenomic virus discovery studies have been conducted for several native and invasive species in Australia including rabbits,[36] foxes,[37] bats,[38] dasyurids (marsupial carnivores),[39] koalas[40] and shorebirds,[41] but expanding the number of species studied and the geographical range of these studies is needed to understand the dynamics of the Australian 'virome'. It is particularly important to conduct unbiased metagenomic screening of domestic species in frequent contact with humans, wildlife at the human–animal interface, and common invertebrate vectors of disease (e.g. mosquitoes, ticks). The latter are known hosts of arboviruses, which represent a significant threat to Australia with over 75 arboviruses having been identified on the continent.[42–44]

As climate change shifts weather patterns across Australia, it will be important to better understand the geographic range of endemic and emerging arboviruses such as Dengue virus, Kunjin virus, Ross River virus (RRV) and JEV,[44] and the ways in which these ranges will respond to climate change. For example, increased flooding in some regions could create conditions ideal for increased migration of viraemic birds and population expansion of arboviral vectors, but, conversely, increased aridity in other regions might actually decrease the risk of disease emergence. Additionally, viruses that already occur across extremely widespread and climatically variable geographic ranges (e.g. Barmah Forest virus, BFV; RRV) might be less affected by anticipated climate change. The dynamics of these viruses cannot be fully understood without widespread monitoring. Sentinel surveillance has been undertaken in northern Australia for decades, especially after the introduction of the Northern Australia Quarantine Strategy in 1989. For example, this monitoring in northern Australia has aimed to identify cases of Dengue virus and JEV,[45,46] including through sentinel surveillance of pigs and mosquitoes.[46] In other regions of Australia, surveillance programs focus more on detection of MVEV and KUNV in sentinel populations of chickens or in opportunistic sampling of mosquito vectors.[47] Despite the strengths of the existing system, there is a strong need to invest further in capability building and infrastructure to expand the surveillance network as the threat of emerging diseases increases. As the genomic era gains momentum in Australia, an increased use of metagenomics-based methods for surveillance and outbreak monitoring

will be of great benefit, although the scale of such a study across a landmass that is largely unpopulated is a great challenge.

Current and emerging threats to Australia

The COVID-19 pandemic most recently brought the risk and consequences of a pandemic into the public consciousness. Subsequent international outbreaks of Mpox and Ebola have also received strong media attention, however, respiratory viruses such as novel strains of influenza, or another novel coronavirus, are far more likely to cause the next global pandemic. Influenza has caused four pandemic events in the past 100 years, and influenza infections are seemingly increasing in frequency.[48] The viruses' wide host range and segmented genome leads to frequent genomic reassortment events, resulting in new strains. At present, the high pathogenicity avian influenza (HPAI) H5 virus is spreading globally through sea birds leading to mass mortality events, as well as causing spillover outbreaks in marine mammals and (occasionally) humans.[49] The virus has not yet been detected in Australia and, therefore, is not considered an imminent risk to human health, but is worth monitoring. It is crucial that there is a widespread capacity for the surveillance of viral threats in Australia, with detection not hampered by inter-jurisdictional differences in laboratory or analytical protocols.[50]

Australia suffers from periodic outbreaks of endemic virus species, many of which are arboviruses. Arboviruses present a significant outbreak threat, particularly in tropical regions and in the aftermath of severe weather events. Of the arboviruses identified in Australia, many are endemic to the tropical northern region of the country, but others are more widespread as host and vector generalist (e.g. RRV, BFV). Outbreak can be common in regions where Australia's population centres are located (Fig. 2).[44] Alphaviruses (e.g. RRV, BFV), orthoflaviviruses (e.g. Murray Valley encephalitis virus, MVEV; Kunjin virus, KUNV)[51,52] and orthobunyaviruses (e.g. Gan Gan virus)[53] are all examples of arboviruses that have caused sporadic outbreaks of disease across Australia. These outbreaks are usually linked to extreme weather events that result in higher-than-normal levels of precipitation, as well as associated abnormal increases in the viral vector populations. For example, an outbreak of MVEV occurred in 2023 across Australia with cases detected in Victoria, Western Australia, Northern Territory, and the first case reported in New South Wales. This outbreak was suspected to have resulted from a pattern of extreme rainfall during the outbreak period.[54] There is also ongoing concern that arboviruses that are not currently endemic in Australia, such as Zika virus, Chikungunya virus and Yellow fever virus, could emerge through widespread outbreaks following introduction by international travellers. These viruses have spread across Asia and South America from their original point of emergence as a consequence of the spread of their vector, the *Aedes aegypti* mosquito. Local transmission of Dengue virus has occurred several times within northern Queensland because of infected travellers returning to the region. At present, within Australia

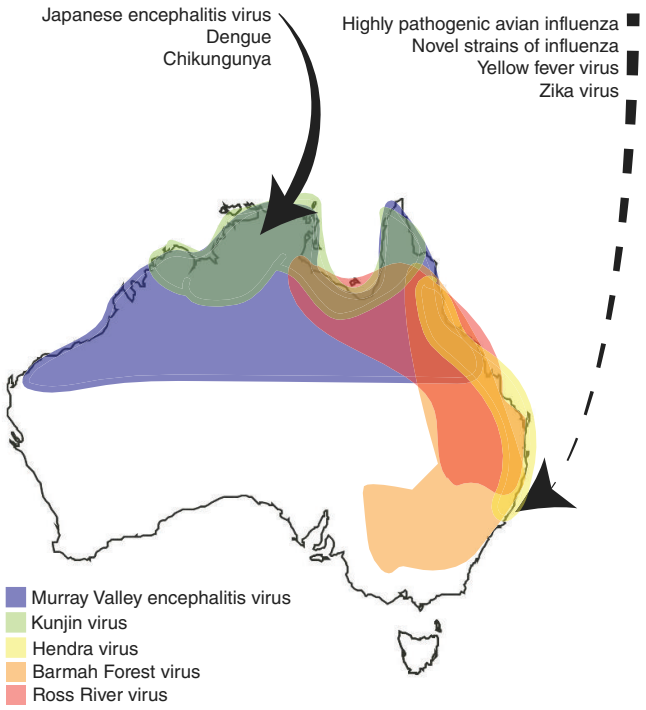

Fig. 2. Endemic and emerging viruses in Australia. The approximate geographic ranges of these endemic viruses are shown in coloured regions, based on where they are most frequently reported in clinical cases or detected in sentinel surveillance. However, it is important to note that these viruses can, and have been, more rarely detected in other areas of Australia, such as Barmah Forest virus being detected as far south as Tasmania.[58] Viruses of predicted emergence risk from neighbouring regions are indicated with a solid arrow. Viruses of predicted risk from broader international regions are indicated with a broken arrow.

A. aegypti is only found in Queensland as an invasive species, and *A. albopictus* (another Dengue virus vector) is established in the Torres Strait but not on the mainland. Previous work has also suggested that other native mosquito species could possibly serve as a vector for viruses of concern (e.g. JEV).[18] Continual monitoring to sample the virome of mosquito species is key to preventing the emergence of devastating arbovirus species in Australia.[44]

There are also other less common viral threats in Australia, including paramyxoviruses. The Hendra virus is likely endemic in the flying fox populations of eastern Australia, and results in sporadic outbreaks within horses and occasional onwards transmission from horses into humans. Additionally, recently a case of Newcastle disease virus caused fatal disease in a child in eastern Australia.[55,56] Likewise, there have also been a small number of spillover events of Australian bat lyssavirus, a relative of the rabies virus, causing disease in humans.[57] However, the spillover events associated with these viruses are incidental, with humans representing a 'dead end host'.

Conclusions

The COVID-19 pandemic exposed Australia's strengths and weaknesses in responding to a global pandemic virus

emergence event. As anthropogenic activities continue to increase the risk of virus emergence, we must be prepared for the emergence of viruses in the future, both within Australia and internationally. For example, increasing extreme weather events are already expanding the range and endemicity of pathogenic arbovirus species (e.g. JEV). The main threats for future pandemics are the emergence and spread of respiratory viruses such as influenza and arboviruses. To prepare for such events, it is crucial that unbiased virus surveillance (e.g. metagenomics) programs be funded across the continent to better understand the diversity of viruses in Australia, the dynamics of virus ecology and the geographic range of viruses with potential to cause disease outbreaks in humans.

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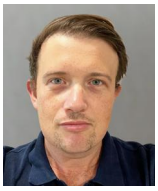

Dr Charles Foster is an early career who investigates big questions in evolutionary biology, including the evolutionary timescale of flowering plants, the evolution of live birth in vertebrates, and virus evolution. Since 2020, Charles has worked as a bioinformatician within the Virology Research Laboratory (University of New South Wales). His research involves developing bioinformatics pipelines for genomic epidemiological surveillance of SARS-CoV-2, high-throughput antiviral resistance testing of human cytomegalovirus, and associations of the human virome with disease outcomes.

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Pandemic lessons learned and future public health strategies

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ABSTRACT

This article explores the significant challenges of the COVID-19 pandemic in Australia since 2020 and reflects on important lessons for preparedness and response to future emergent infectious diseases or pandemics. It highlights the importance of One Health as a framework for pandemic preparedness; near-real time surveillance data to inform responses; and the critical place of equity considerations in planning, preparedness, response and recovery. The role of crisis communication and engagement is explored, noting the significant place of local engagement, informed by local epidemiological data and local communication needs and priorities of diverse communities.

Keywords: communication, COVID-19, equity, One Health, pandemic.

In this, the fifth year of the COVID-19 pandemic, it is worth reflecting on some of the important lessons informed by global and local experiences. There has been some debate over whether we are still in a 'pandemic phase' and – if so – whether COVID-19 remains 'exceptional'. The more pertinent analysis here is on the ongoing impact, rather than the semantics of what an ongoing pandemic might mean.

The Australian Bureau of Statistics reports that in 2023 to 30 September, there were 137,048 deaths in Australia.[1] This is 5.7% less than for the same period in 2022. Although this is positive in terms of the ongoing impact of COVID-19 on Australian mortality, it is still 9.9% above the baseline average. This therefore represents an excess mortality of 12,377 over baseline. Although this may be due in part to deferred care, reduced illness screening and other direct and indirect health impacts of the pandemic, there is a high likelihood that COVID-19 is responsible for the majority of excess mortality in Australia.[2] Nonetheless, the excess mortality has shown a substantial correlation with COVID-19 hospitalisations and reported deaths; now tending towards peaks 4–6 months apart[3] with variation according to circulating strains.

In addition to this substantial increased burden of mortality, there is growing concern at the potential health, social and economic costs of Long COVID. The myriad post-acute sequelae[4] represent a significant societal cost in medical terms, but the more substantial burden may well be in the chronic symptoms and disability borne by survivors and those who love and care for them. The potential impact on cognitive function[5] alone should be a cause of concern as the population-level impacts in the working-age population will be multi-dimensional, causing suffering and affecting productivity.

The COVID-19 pandemic certainly challenged the global assumptions of pandemic preparedness, with an understandable but unfortunate bias towards novel influenza as a potential cause of a pandemic. Countries that had direct experience with another coronavirus (e.g. SARS in 2003) did comparatively better on performance measures[6] than those that did not, despite the higher baseline risk in these countries. It is notable that many of the better-performing countries enacted early, robust public health measures, but that not all of these countries pursued 'aggressive suppression' in the way that Australia, New Zealand, China, Taiwan and others did.

The enormous economic cost, mental health and social burden of aggressive suppression policies must be interrogated and understood in order to inform future approaches. Although agent-based modelling has been extremely useful in informing responses in Australia and the potential policy settings in differing scenarios,[7] it must be understood that in an 'effective elimination' approach, the optimal approach is highly contingent on the timing, feasibility and social acceptability of such measures. To quote Dr Mike Ryan, executive director of the World Health Organization's Health Emergencies Programme, when it comes to infectious disease emergencies, 'speed trumps perfection' and 'the greatest error is not to move'.[8]

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In settings where border closures and mass quarantine settings are in place, early, robust public health measures tip the scales significantly towards an elimination (COVID Zero) approach. Such early action leads not just to minimal morbidity and mortality, but to significantly averted costs and an overall less restrictive response when the full period of pre-vaccination response is considered. New Zealand, as a prime example, had overall lower average stringency of public health measures in the pre-vaccine phase, despite strict lockdowns as part of the initial pandemic response.[9] It remains a country with one of the lowest excess mortality rates globally, even 4 years into the pandemic.[10] However, where this is not feasible or where social licence is not supportive, then the most effective, sustainable mitigation strategies must be employed. The challenge is in the sustainability of such measures, especially when more costly or where they impose significant constraints on normal activity.

In this phase of the pandemic, as successive viral variants become more transmissible, public health and social measures are significantly harder to maintain and arguably less effective in reducing the burden of illness. This requires a strong focus on the key interventions that reduce severity of illness and level of transmission. Vaccines for COVID-19 were explored, developed, tested and mass produced in an astonishingly short period[11] given the 'usual' timelines for vaccine development and deployment. That is a testament to the incredible global effort that was focused on this profound challenge, but also to the decades of research in vaccine technology, including mRNA technology, that formed the foundation for such exploration and achievement.

Although vaccination (and, to a degree, antiviral treatments) have been the most significant intervention to reduce the potential burden of mortality in the pandemic,[12] it has manifested an all-too-familiar challenge of public health interventions; that of inequity.

This author believes that future public health strategies must therefore consider four core pillars of pandemic planning, preparedness, mitigation and response: a 'One Health' approach; a health equity lens; a sustainable, systems approach to reduction in transmission risk; and an effective community engagement approach to crisis communication.

The World Health Organization defines One Health as 'an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems'.[13]

The importance of such an approach is recognised in the establishment of the Australian Centre for Disease Control (ACDC) where there is an explicit reference to building capacity in health security and One Health.[14] This framework is critical for planning and preparing for emerging infectious diseases for a number of reasons, including that three-quarters of new or emergent infectious diseases in humans are zoonotic.[15]

The capacity for early identification and response to diseases with epidemic or pandemic potential – whether in animal or human populations – is therefore a core requirement for Australia's national surveillance system. Of course, such diseases will not always arise in Australia; in fact, most will arise elsewhere in the world, especially at the human–animal interface where global health security must focus. Nonetheless, a robust, near-real-time surveillance system in Australia is a necessary pillar of pandemic preparedness. It remains the case that 4 years into the COVID-19 pandemic there is no national case definition for hospitalised cases, making real-time comparisons of prevalence and severity across jurisdictions effectively impossible. The ACDC must have nationally standardised minimum data collection requirements and case definitions as one of its first, early 'wins', allowing for a true national picture to help inform responses that are evidence-based, proportionate and timely.

An equity lens is crucial for several reasons. The Grattan Institute has already highlighted the significant health gaps that played out through the pandemic.[16] The most at-risk populations for COVID-19 infection, hospitalisation and dying were also those in the lower socio-economic strata and populations born overseas. In particular, older population cohorts, especially residents in Aged Care settings, were significantly represented in the burden of morbidity and mortality. Such populations therefore require a concerted focus and additional support through the pandemic, but this is always profoundly challenging through the early, emergency phase of a pandemic and therefore the substantial work of engagement and policy levers with a focus on minimising inequity must occur in the pre- or inter-pandemic phases.

The other critical issue of equity is in access to, and uptake of, vaccines. Similar population-level disparities emerged through the pandemic[16] but there were many examples of achieving high vaccination coverage and geographic and broader equity in coverage in Australia, despite the economic and cultural differences in target cohorts. A study of vaccination coverage in south-east Melbourne demonstrated the ability to significantly close the equity gap in vaccination coverage with multi-layered interventions.[17]

The challenge with behavioural interventions is the sustained effort that is required and fragile social licence with certain interventions. It is thus critical to explore system-level interventions that do not require significant behavioural impetus. Clean indoor air, through natural and augmented ventilation, is rightly being explored to this end, including by the US Office of Science and Technology Policy.[18] The ability of indoor air ventilation and germicidal UV to reduce transmission risk of COVID-19 and many other respiratory pathogens is increasingly being demonstrated.[19] There is an urgent need now to explore further the cost-effectiveness and cost–benefits of such interventions.

Finally, there is a clear need for communication and engagement approaches that support social cohesion, trust in public health measures and sustained behaviours that protect health. The COVID-19 pandemic was a demonstration of the need to utilise better crisis communication principles,[20] including open, honest communication; acknowledging uncertainty; speaking with compassion and being responsive to changing circumstances and community engagement needs. Future crises – of whatever kind – will require the same principles to be applied if we are going to be truly accountable to the community. In an era of rising mis- and disinformation, and artificial intelligence enabling of 'deep fakes' and the occasional production of 'hallucinations', there is an urgent need to improve science literacy and provide individuals with the tools, such as 'pre-bunking' and strengthening critical

thinking, to counter false information.[21] The next pandemic demands it.

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Biography

Professor Brett Sutton is Director of Health & Biosecurity at CSIRO, Australia's national science agency. He is a qualified public health physician, with extensive experience and clinical expertise in public health and communicable diseases, gained through experience in Government, emergency medicine and field-based international work. Prior to CSIRO, he held the role of Victoria's Chief Health Officer together with the role of Victoria's Chief Human Biosecurity Officer.

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Microbiology Australia

External quality control processes for infectious disease testing

Wayne Dimech[A], Guiseppe Vincini[A] and Belinda McEwan[B]

Introduction

Historically, serological testing for infectious diseases was performed using biological assays such as complement fixation or haemagglutination inhibition. These assays utilised the agglutination or haemolysis of red blood cells as biological indicators for the presence or absence of antibodies.[1] Generally, a four-fold difference in doubling dilution titres was required to consider a significant difference in antibody levels. Over the 1990s, with the advent of enzyme immunoassays (EIAs), testing for infectious disease antibodies moved away from biological assays, which were labour intensive and difficult to control, to microtiter plate EIAs and then to automated platforms.[1,2] The output of these tests is reported in a unit of measure calculated from the intensity of the signal produced by the reaction, be it colorimetric, immunofluorescent or chemiluminescent. This signal, often referred to as a signal to cut-off (S/Co) is an arbitrary unit based on the comparison of the signal produced by the patient sample compared with a cut-off determined by the manufacturer e.g. a multiplier of the negative control signal or the mean value of particular calibrators. The 'cut-off' value for these assays effectively becomes the assay decision point, separating the populations of samples with the target analyte from those that do not contain the target analyte. Whereas the S/Co or other arbitrary unit will generally increase as the amounts of antibody increases in sample tested, the test system is not measuring the quantity of antibodies present, it measures the amount of binding of the antibody in the patient sample with the antigen on the solid phase of the assay.[1–3]

Testing for infectious diseases using immunoassays gradually became available on high-throughput immunoassay platforms that also test for clinical chemistry markers. In more advanced countries, infectious disease testing has moved away from the microbiology laboratory into 'core laboratories', where the instruments and associated processes, including the quality control processes, are managed using a singular system within the same laboratory, typically the traditional approaches applied to clinical chemistry testing. However, testing for inert chemicals such as glucose and potassium measure the amount of analyte in the patient sample. In these situations, the test system is calibrated to a standard, often an international standard, and the results are expressed in SI units. This lends itself to certain statistical methodologies. By contrast, the arbitrary S/Co result obtained in infectious disease testing is influenced by a range of factors relating to the antibodies being detected including their avidity or affinity, genotype or subtype of causative agent, stage of disease progression, immune status of the patient, and factors relating to the assay itself such as target antigens, antibodies utilised in the conjugate, and chemistry applied to create and detect the signal.[4]

Quality control

The use of a quality control (QC) sample is a requirement for laboratories accredited to ISO 15189 and is defined in the standard as an 'internal procedure which monitors the testing process to decide if the system is working correctly and gives confidence that the results are reliable enough to be released' (section 3.11, p. 3[5]). Further in the ISO 15189 document it states, 'The procedure should also allow for the detection of lot-to-lot reagent and/or calibrator variation of the examination method.' (section 7.2.7.2(a), p. 25[5]). The National Association of Testing Authorities (NATA) ISO 15189 Standard Application Document (SAD) refers to QC processes as 'A system must be established for the long-term monitoring of internal quality control results to assess method performance' (section 5.6.2, p. 11[6]). Frequently, laboratories will interpret the standard to suggest that the use of a kit control is adequate to fulfil the requirement.

Kit controls

Kit controls in infectious disease testing have the purpose of validating the test. Generally, kit controls are tested, and the results are accepted prior to testing patient samples. The manufacturer provides the kit controls and associated acceptance criteria. These acceptance criteria have been developed by the manufacturer in pre-market clinical trials and results within the established range can be taken as evidence that the test kit is performing as expected by the manufacturer and the sensitivity and specificity claimed by the manufacturer can be assured. It is often pointed out that the acceptance range for kit controls are wide. This is in fact the case because infectious disease serology assays tolerate significant changes in signal before the clinical sensitivity and specificity is compromised. Note that historically biological assays allowed a four-fold change in dilutions before

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a significant difference was confirmed. Kit controls are required to be tested when stated in the manufacturer's instructions for use (IFU). All infectious disease assays are listed on the Australian Registry for Therapeutic Goods (ARTG) as class 3 or class 4 *in vitro* diagnostic devices (IVD).[7] Laboratories reporting clinical results are required to follow the IFU without deviation. Any modification to the IFU, such as not using a specified kit control, means the assay is being used 'off licence' and becomes an 'in-house IVD' which must be registered as such with the Therapeutic Goods Administration (TGA). In cases where the manufacturer's IFU does not require the testing of the kit control, their use is highly recommended as best practice. Kit controls should not be replaced with third party controls but utilised in conjunction with them.

Third party controls

The ISO 15189 standard states 'To enable this, (the ability to detect lot to lot variation) the use of third-party IQC material should be considered, either as an alternative to, or in addition to, control material supplied by the reagent or instrument manufacturer' (section 7.2.7.2(a), p. 25[5]). Whereas the kit controls are designed to validate the assay at the time of testing, they are not designed to monitor the performance of the assay over time. Generally, kit controls are not sensitive to changes in the test system. Well-designed third-party controls are IVDs manufactured by companies other than the test kit manufacturer and are designed to monitor variation.[4,8] These controls should have a reactivity at a level that can detect variation. The ISO 15189 standard states 'the IQC material provides a clinically relevant challenge to the examination method, has concentrations levels at or near clinical decision points' (section 7.2.7.2(b), p. 26[5]). Immunoassays do not have a linear dose response curve. That is, as the amount of analyte being detected increases, it is expected that the signal will increase proportionally. In most immunoassays, the dose response curve is sigmoidal. Initially as the amount of analyte increases there is only a small increase in signal. As the analyte concentration increases, the curve becomes linear until such time that all or part of the components are exhausted, after which the curve plateaus. The third-party controls must therefore be reactive in the linear part of the curve to be effective in detecting variation, and the linear part of a curve may not necessarily be close to the cut-off of an assay.[1]

The NATA SAD states, 'Numerical QC results should be presented graphically to assist in the early detection of trends' (section 5.6.2, p. 11[6]). Infectious disease testing has a numerical value (the S/Co or other arbitrary unit), noting that these numbers are not a measure of an amount of antibodies, rather a measure of binding activity. However, they can be plotted on a Levey-Jennings chart and effectively monitor variation in the test system. If the supplier of the third-party control has minimal lot-to-lot variation, the results of multiple lots of the same third-party QC can be used to monitor the assay over many years, providing the laboratory good insight into the assays long-term precision and bias.[9]

The use of a third-party QC optimised for the assay being monitored is highly encouraged. It serves a different, but complimentary, purpose to the kit controls and laboratories should use both the kit control and the third-party control. At a minimum the use of either the kit control or third-party control is mandatory for laboratories accredited to ISO 15189.[5]

Acceptance ranges for third party controls

Guidance on how QC results are managed is limited. The NATA SAD states, 'A system must be established for the long-term monitoring of internal quality control results to assess method performance' (section 5.6.2, p. 11[6]). The National Pathology Accreditation Advisory Committee (NPAAC) Requirements for Quality Control, External Quality Assurance and Method Evaluation document, quality control section states 'For quantitative assays, target values and SDs must be determined using laboratory data' but does not specify how these ranges are determined.[10] Traditionally, clinical chemists have used the mean ± x standard deviation (s.d.) of a small data set (e.g. 20–30 results) and applied Westgard rules to identify unexpected variation.[11,12] As infectious disease testing moved from the microbiology laboratory to the 'core laboratory', it is unsurprising that these traditional methods have been applied to the S/Co or arbitrary values expressed by the immunoassays. However, it has long been anecdotally recognised, and recently published, that infectious disease testing experiences significant reagent lot-to-lot variation and the use of traditional QC methods causes unacceptable numbers of false rejections.[4,13] Therefore, when an acceptance range based on 20–30 QC results is used to establish the QC acceptance range, frequently new reagent lots cause the QC to be out of range and therefore rejected. The laboratory is therefore faced with a dilemma. Do they reject the reagent based on the QC result, noting that the kit controls are usually within the manufacturer's acceptance criteria, indicating no change in sensitivity or specificity? Or do they re-calculate the range using the next 20–30 results, with the knowledge that the introduction of a new reagent lot will repeat the same situation? The laboratory would also need to justify why it is appropriate to release patient results using multiple acceptance criteria over time.

It should be noted that recalculating the mean and s.d. on a new reagent lot only re-establishes the imprecision of the assay. However, the change in reactivity of the QC is not due to a change in imprecision, but an introduction of bias caused by the new reagent. Recalculation of the mean and s.d. therefore ignores the root cause of the change and does not address the fundamental question of how much variation due to reagent lot change is acceptable.

Irrespective of the method utilised to establish the acceptance range for each QC sample, the methodology must be based on scientific evidence using data from the same test process being controlled, rather than assuming commutability of methodology. As the ISO 15198 standard states, when selecting a QC methodology, 'The intended clinical application of the examination should be considered, as the

performance specifications for the same measurand may differ in different clinical settings' (section 7.2.7.2(a), p. 11[5]). This evidence should be made available to an auditor when requested.

Infectious disease specific QC requirements

The ISO 15189 standard does not specify which quality control methodologies should be employed.[5] Like most standards designed for a broad set of disciplines, it is not prescriptive. This is also the case for the NATA SAD and NPAAC Requirements for Quality Control, External Quality Assurance and Method Evaluation documents, unlike the UK-equivalent Standards for Microbiology Investigations, Quality Assurance in the Diagnostic Infection Sciences Laboratory document, which implies the use of traditional methods including Westgard rules for use in infectious disease serology.[14] This UK standard, however, has been recently modified to acknowledge that traditional methods are not perfect and thus includes alternative methods of QC including the use of QConnect limits.

The NATA SAD does provide additional discipline-specific QC requirements, including cartridge-based assays, chemical pathology, cytology, haematology and histopathology. To address the points relating to the provision of QC methods for infectious disease testing raised above, an additional infectious disease disciple-specific section will be added to the NATA SAD. These clauses will be included in the accreditation of medical testing laboratories in Australia.

The inclusion states:

- Controls provided by the manufacturer (kit controls) must be used if the manufacturer's instructions for use (IFU) state that their use is required.
- If the use of kit controls is not required by the manufacturer's IFU, then a laboratory must use at least one of a kit control or a third-party external quality control (EQC) to validate the test each day the test is used.
- Use of both kit controls and EQC is recommended. Where suitable EQC specimens are available, their use in maintaining QC is recommended.
- If the laboratory uses the kit controls to validate the test, they must use the validation rules specified by the manufacturer.
- If the laboratory uses EQCs to validate the test, the EQC must be validated by the laboratory for use on that test.
- The laboratory must have a documented method for establishing acceptance criteria for an EQC based on scientific evidence that is validated using infectious disease data.
- The laboratory must have documented procedure for when the controls are outside the established acceptance criteria.

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Disclosure statement. Belinda McEwan in the ASM-authorised representative on the National Association of Testing Authorities and Human Pathology Accreditation Advisory Committee of the National Pathology Accreditation Advisory Council.

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Parliamentary Friends of Science

Victoria Wansink[A]

Hosted by Science and Technology Australia (STA), the Parliamentary Friends of Science breakfast, held on 30 November 2023 at Parliament House, Canberra, was attended by a diverse mix of parliamentarians, STEM-sector leads and experts, and senior public servants. Representing The Australian Society for Microbiology (ASM), Dr Victoria Wansink (Director Microbiology, ACT Health) listened to the four-person panel share their thoughts on the challenges faced by the STEM workforce in Australia. The aspects that the panel focused on, included preparing the next generation of scientist for careers that may not even exist currently, the importance of diversity within the workforce (including the emphasis on varieties of work–life experiences, career pathways, (cross) training, etc.) and flexibility in multi-faceted, multi-directional careers (a 'London Underground' of opportunities).

The many conversations during the networking opportunities, provided before and after the official proceedings, highlighted the variety of attendees, but all displayed a genuine interest and curiosity in STEM and linkages with government processes and policy developments. The ability for the STEM professionals in the room to engage with parliamentarians, policy makers and other senior public servants is crucial for the demystifying of the STEM fields, encouraging critical thinking and curiosity, as well as ensuring that messaging relating to STEM being present everywhere it is needed.

I had many conversations about the field of microbiology (mainly kickstarted by the dress I managed to find for the event, see Fig. 1), as well as more general science, communication and research-funding topics. The warm welcome I received from STA organisers was a credit to Peter Traynor's previous networking with the organisation and STA was pleased that

Fig. 1. The dress that kickstarted conversations about microbiology.

The ASM was again represented for the event. The time (1 h) flew by and, before we knew it, it was time to be escorted back to the public entrance and start a regular workday.

Personally, I felt very honoured to represent The ASM and its members at this event. It was an inspiring look inside Parliament House and at how small, short gatherings can spark insightful and respectful conversation to educate and inspire. It was a unique (yet somewhat daunting) way to start the day, but, should the opportunity arise again, I would definitely enjoy attending (and may even stop to look more closely at some of the beautiful surroundings of Parliament House)!

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Call for Editorial Board members

Microbiology Australia seeks ASM members for its Editorial Board. This can be a good opportunity to provide expert advice in the guidance of our journal. Early Career Researchers as well as older members are welcomed. We would especially like to see new members with interests or expertise in Mycology, Veterinary Microbiology and Public Health. The Editorial Board meets four times per year by teleconference.

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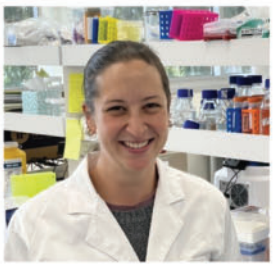

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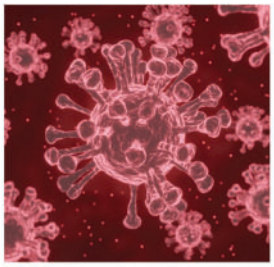

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